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CHEMISTRY OF HEART FAILURE

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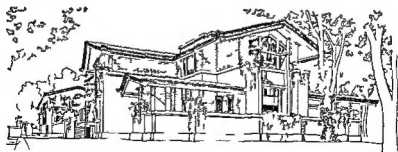
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and
Natalie Titus Bertonis

PREFACE

In this monograph we have attempted to gather in a few pages some of the extensive information pertinent to the mechanism of contraction in the normal and failing heart and the mode of action of digitalis. The book has been written so that the medical student, clinician and cardiologist with an elementary knowledge of physics and chemistry can learn of the many advances in this field in a few leisure hours. Sufficient references on the subject are included so that the ambitious student may delve further if he chooses.

We feel the book will also appeal to the chemist and physicist who are curious about the development and application of their own subject in another very important field.

Every attempt has been made to give proper credit to first discoveries. Important contributions which have been omitted represent gaps in the authors' capacity to find or summarize all of the relevant material.

W C HOLLAND
R I KLEIN

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CHEMISTRY OF HEART FAILURE

Chapter I

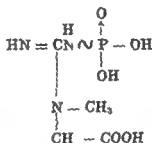
INTRODUCTION

In order for the heart to perform its necessary function of propelling blood through the circulatory system it must contain the necessary metabolic machinery for the release, conservation and subsequent transformation of chemical potential energy of foodstuffs into mechanical work. During the past fifty years many advances have been made in our understanding not only of the chemical mechanisms involved but also of the physico-chemical events that underlie the conversion of chemical potential energy into mechanical work in both cardiac and skeletal muscle. In fact we now have good evidence that one of the principal and final causes of the important forms of congestive heart failure is in essence a failure of the utilization of chemical potential energy.

These advances have been made possible by application of techniques of classical organic chemistry and physics and also by the development of muscle models such as the glycerinated soas preparation of Szent Gyorgy.¹ The latter are of great importance because they stand half way between whole muscle which was the object of classical physiology and the protein and enzyme systems which were studied in the chemical laboratory. In the present chapter we would like to review briefly these major advances. In this way the reader may approach the remaining chapters with an integrated view of events associated with the contraction of the normal and failing heart.

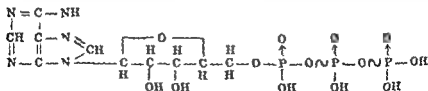
The history of the chemistry of muscle contraction has been characterized by a relatively slow evolution of concepts interspersed with periods of revolution in which existing theories were hastily abandoned and new avenues of thought explored. It was early evident that the contractile activity of heart and skeletal muscle

depended upon oxygen consumption and metabolism of foodstuffs such as carbohydrates. By the late twenties it was realized that the relation of these metabolic processes to the contractile process was an indirect one. Until this time it was believed that lactic acid was the agent that drove the contractile machinery. Lundsgaard's discovery³ that contraction persisted after all respiration and lactic acid formation was eliminated dispelled this idea quickly. He gave evidence that the chemical event supporting contraction was the breakdown of phosphocreatine. Lactic acid production occurred after contraction was over.



Phosphocreatine

A few years later Lohmann⁴ proposed that phosphocreatine was only indirectly involved and the essential role was assigned to adenosine triphosphate (ATP). ATP was broken down to adenosine diphosphate (ADP) which in turn accepted phosphate from phosphocreatine. In this manner ATP was thought to energize the contractile mechanism.



Adenosine triphosphate

As evidence continued to accumulate it was soon realized that the prime function of glycolysis and respiration was to furnish ATP by synthesis from ADP and phosphate. The processes of respiration

were relegated to the recovery phases of muscle activity. Mechanical work was achieved at the expense of chemical bond energy of ATP with phosphocreatine acting as an additional reserve or source of phosphate bond energy. Respiration and glycolysis then resynthesized ATP to replace that which was used up.

By 1939 a reasonably consistent picture of the energetics of muscle contraction had emerged. The problems of the nature of the mechanochemical coupling whereby energy obtained from the breakdown of ATP drove the contractile machinery was left unanswered.

In 1859 Kuhne discovered that large quantities of a viscous protein could be extracted from muscle by strong salt solutions. He named this protein myosin. However it took more than 75 years for the full significance of this fundamental observation to be appreciated because of the inadequate techniques of the time.

Later optical studies⁸ of flowing solutions of myosin revealed a birefringence which suggested it consisted of elongated molecules. This made myosin a likely candidate for the structural component as well as the working substance of the myofibril. With knowledge of the chemical nature of the working substance and of the energy source ATP the question arose as to whether a definite relation existed between the two entities. It was indeed significant when Engelhardt and Ljubimowa in 1939 discovered that myosin was an enzyme, adenosine triphosphatase, which catalyzed the breakdown of ATP to ADP. From these studies the concept arose that myosin was a contractile enzyme deriving its energy from the hydrolysis of ATP.

At about the same time Needham and coworkers⁹ showed that myosin underwent certain remarkable reactions with ATP. The birefringence of myosin solutions disappeared or decreased on the addition of ATP. Myosin threads prepared by squirting myosin into aqueous solutions contracted with ATP. It was apparent that ATP affected the physicochemical properties of myosin and at the same time was enzymatically hydrolyzed to ADP.

These observations were reinterpreted in a different light when Szent Gyorgyi and collaborators¹⁰ showed that myosin was in

reality a complex of two proteins actin and myosin which they called actomyosin. From their findings the idea arose that during the resting state actin and myosin were kept dissociated and that upon stimulation actin combined with myosin to form actomyosin. Upon the adsorption of ATP actomyosin went into a contracted state. The molecular event was visualized as a folding or coiling of a polyelectrolyte strand containing actomyosin.¹⁰ It is important to note that in this proposed mechanism contraction was energized by the adsorption and not the hydrolysis of ATP. Questions concerning the localization of these proteins in the muscle fiber and the relation of the chemical events during contraction to the known changes in muscle morphology were still not answered.

Since the late nineteenth century it was known that muscle striations were an important clue to the mechanism of contraction. In fact the changes in striations accompanying contraction were accurately and clearly described by the nineteenth century microscopists¹¹ but unfortunately no coherent theory of muscle contraction emerged. These significant observations were neglected probably because of the rapid progress being made in muscle chemistry and energetics of contraction.

By 1910 muscle chemistry had reached a stage in development where it was ready for correlation with structure. Furthermore the mechanics and heat production of muscle had also developed sufficiently to be linked with other lines of information on the contractile process. By 1915 there was good evidence that the process which turned on contraction was a reduction of the resting membrane potential and that calcium in some as yet unknown manner played a crucial role in this process of excitation-contraction coupling.

By 1953 the techniques of thin sectioning for electron microscopy as well as those for the specific extraction of actin and myosin from intact muscle fibers were developed to a high degree. Using these methods the Huxleys and their collaborators¹⁻³ were able to attack the problem of localization of the structural proteins in the muscle fiber. They found evidence that the anisotropic or A band was due to the presence of thick filaments containing myosin. They were also able to show that a second set of filaments thinner

than the first and containing actin extended from the Z membrane through the isotropic or I band into the A band. There the thin actin filaments interdigitated with the thick A band filaments. From these studies and a number of others they proposed a rather simple and radically different model of muscle contraction. They postulated that the wave of depolarization induced changes which permitted the thin I filaments to slide past the stationary and thicker A filaments and shortening ensued. This process was pictured as being energized by the breakdown of ATP.

As a consequence of the many advances in our knowledge of the chemistry of the normal contractile process it was only natural that the new techniques and ideas were applied early to a study of the nature of the defects in congestive heart failure.

Much of what was known about the nature of heart failure was obtained from hemodynamic and mechanical considerations and was embodied in the Frank-Starling Law of the heart. This principle states that the energy of contraction of the heart is a function of the initial length of the myocardial fibers—that is, the diastolic volume of the heart. Starling showed that contraction energy increased up to a certain optimal length but with further lengthening of the fibers the energy of contraction diminished. He felt that this resulted from the fiber being stretched beyond a critical length. According to the views of Starling, congestive failure could be thought of as resulting from excessive stretch of the muscle fibers.

It has become increasingly apparent that only in the case of failures resulting from anoxia, vitamin deficiencies and hormonal imbalances can a metabolic defect be demonstrated. Here a failure of energy release is reasonably clear-cut. On the other hand, in failures resulting from chronic hypertensive and valvular disease in humans as well as in spontaneous failures of the heart-lung preparation, the metabolic patterns appear to be perfectly normal. The evidence available to date strongly suggests that the basic defect in these conditions is a failure to transform available metabolic energy into mechanical work.¹⁴ It is only the latter type of failures that respond effectively and efficiently to the digitalis glycosides.

Several questions arise if we accept the concept proposed by

Frank and Starling that in final analysis congestive failure results from excessive stretch of the myocardial fibers. Does excessive stretch lead to the development of abnormal contractile proteins, a failure of excitation-contraction coupling, the loss of essential metabolites or electrolytes such as K and Ca, or the partial or complete withdrawal of the thin actin filaments from the A band where they are normally interspersed among the thicker myosin filaments? The answers to these questions are not available at the present time.

There is evidence that Cr ions are somehow involved in the genesis of congestive failure. It has long been known that digitalis can in part substitute for Ca ions, and that Cr ions are required for the beneficial effects of this drug. Furthermore it has recently been shown that the digitalis glycosides¹⁴ increase the available myocardial Ca ions either at sites in the membrane or in the myoplasm irrespective of any effect they might have on metabolic reactions. Many of the problems presented above will be discussed in some detail in the following chapters.

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Chapter II

ELEMENTS OF THERMODYNAMICS

Introduction It is very difficult to grasp the full significance of the chemical mechanisms involved in the release and transformation of chemical potential energy of foodstuffs into work by the heart without having an appreciation for the concept of energy. Historically, force is the basic concept in the consideration of energy. Force is measured in terms of acceleration imparted in unit mass. Work is defined as the action of force over unit length and energy as the ability or capacity to do work. Energy appears in various forms: heat, light, kinetic, mechanical work, electrical potential and chemical.

The First Law of thermodynamics states that energy can change its form but not its quantity. In thermochemistry the chemical potential energy of a given substance is expressed quantitatively as its heat equivalent (in ergs, joules or calories). The Second Law of thermodynamics places restrictions on the exchange and transformation of this heat equivalent. Stated simply, heat cannot be transformed into work unless temperature differences exist in the system, as is the case with a heat engine. In isothermal systems such as the living cell, where no temperature differences exist, exchange and transformation of energy can occur without wasteful loss as heat only by a process of coupling between the reacting components. Furthermore, the exchange can only occur with high efficiency between substances of comparable chemical potential.

Obviously, the biological system is not like a heat engine and cannot convert heat and pressure changes into work. For instance, the utilization of energy from the chemical reaction



must be achieved by reactions designed to trap the energy released. These reactions must be of such a nature that large quantities of chemical potential energy are broken up into smaller biologically useful packets of the order of 10 to 12 kcal to accommodate the dimensions of the energy currency of the cell.

The chemical reactions involved in this constant flow of energy in biological systems are catalyzed by enzymes or biocatalysts. A study of energetics provides an intimate knowledge of metabolic pathways and life processes. Furthermore, the principles of energetics are of value in predicting whether or not a particular reaction is thermodynamically possible and the extent to which a reaction may proceed. Enzymes only hasten the attainment of equilibrium and cannot catalyze energetically unfavorable reactions nor change the final equilibrium ratio of reactants to products.

Concept of Free Energy In chemistry it is well known that all chemical compounds contain potential energy. This energy is in the form of forces that hold atoms together. When chemical substances react, part of that energy may be converted into work in other chemical systems. It is therefore extremely important to know the amount of work that can be obtained from the reactions of a given compound. It is known from thermodynamical considerations that at a given temperature only a portion of the total energy of a system may be used for work. This fraction available for work is known as the free energy. It can be defined as the maximum amount of work that can be obtained from a system. The symbol F is used to denote this quantity.

In actual practice, not all of the maximum available energy of any particular system is used for work; some of it is wasted as friction and heat. The efficiency of the system is a measure of the fraction of the maximum available energy that is converted to work. Most biological systems have efficiencies of the order of 30 to 60 percent. Thus, if it were possible to carry out a reaction under ideal conditions, the work that reaction could do is the free energy. For any specific system, we cannot specify the absolute value of free energy. However, if the system undergoes a change, there will be a change in free energy, ΔF , which can be measured.

That fraction of the total energy of a system that is unavail-

able for work is called entropy and has been given the symbol S . Again only changes in S , ΔS , can be measured. There is a relatively simple relation between the total energy, the free energy and entropy of any given system

$$H = F + TS$$

or when the system undergoes change

$$\Delta H = \Delta F + T \Delta S$$

ΔH is a change in internal energy or for practical purposes the total energy and T is the absolute temperature at which the transformation occurred. From these considerations it can be seen that of the total energy change accompanying a chemical transformation at a given temperature ΔH is that available for work and ΔS is that unavailable. It is evident from the equation that the sign and size of ΔF will reflect contributions from both the changes in internal energy and the changes in entropy during the transformation.

Changes in free energy ΔF , other than providing a measure of the maximum amount of energy which may be obtained as work in the course of a material change, also provides a quantitative indication of the potential ability of the chemical substance to undergo transformation. Depending upon the sign (\pm) of free energy change, reactions may be classified as exergonic or endergonic. Exergonic reactions have a $-\Delta F$ and tend to proceed spontaneously, such as hydrolytic reactions. On the other hand, an endergonic reaction with a $+\Delta F$ must be coupled with an exergonic reaction in order to proceed spontaneously. Many biosynthetic reactions fall into this group.

Due to the difficulties in estimating changes in entropy ΔS , changes in free energy can be determined by other methods such as from equilibrium data by the following relation

$$\Delta F = -2.303RT \log K$$

where R is the gas constant (1.98 calories per degree per mole), T the absolute temperature and K the equilibrium constant for the reaction.

Equilibrium and Mass Action The above expression leads us immediately to a discussion of the Law of Mass Action. In a general

way the law of mass action is an expression of the fact that (1) the rates of chemical reactions are proportional to the active masses of the substances reacting and (2) in a reversible reaction the ultimate position of equilibrium is determined by the ratio of the active masses of the reaction products and reacting substances. In a reaction such as



at equilibrium the following relation holds

$$\Delta F = \Delta F^\circ + 2.303RT \log \frac{[C][D]}{[A][B]}$$

where the concentrations are those at equilibrium. The ratio of the concentrations of products to reactants is known as the equilibrium constant. The term ΔF° is a constant for the reaction and is called the standard free energy change. When the reactants and products are all present in their standard states or unit concentrations (1 atmosphere pressure for gases, pure liquids or solids and one molal for solutes) $\Delta F = \Delta F^\circ$. The necessity for having such a constant for any reaction is apparent since we cannot record ΔF s for all possible concentrations of reactants and products.

In a reaction which has reached equilibrium the reactants and products are exchanging molecules at the same rate. Therefore $\Delta F = 0$ and the equation becomes

$$\Delta F^\circ = -2.303RT \log K$$

It should be apparent from this relation that the sign of ΔF° is a measure of the spontaneity of chemical change. For example, if K is greater than 1 (more products than reactants) the log of K is positive and ΔF° is negative. On the other hand, if K is less than 1 the log of K is negative and ΔF° will be positive. When $K = 1$ the log of $K = 0$, $\Delta F^\circ = 0$ and the reaction is freely reversible with no net accumulation of reactants or products.

Coupling Mechanisms At first glance it might appear that a reaction with a $+\Delta F^\circ$ cannot occur at all. Many such reactions, however, are known to occur in nature. The reason they occur lies in the fact that living systems provide a coupling mechanism whereby energy of an exergonic reaction (that is, one with a $-\Delta F^\circ$)

able for work is called entropy and has been given the symbol S . Again only changes in S , ΔS , can be measured. There is a relatively simple relation between the total energy, the free energy and entropy of any given system

$$H = F + TS$$

or when the system undergoes change

$$\Delta H \approx \Delta F + T \Delta S$$

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Equilibrium and Mass Action The above expression leads us immediately to a discussion of the Law of Mass Action. In a general

nine phosphate in invertebrates. The mechanism of exchange is known as the Lohmann¹ reaction



This reaction has a very small ΔF° which means that the energy of ATP exchanges smoothly and with high efficiency

Concept of 'Energy Rich' Bonds It will be noted that ATP contains pyrophosphate bonds. Such chemical bonds liberate relatively large amounts of free energy upon hydrolysis. Lipman referred to these bonds as high energy bonds and designated them with the symbol \sim in contradistinction to those bonds which release relatively small amounts of free energy upon hydrolysis such as simple phosphate esters. The symbol is now also used for other high energy bonds not involving phosphate. In Table I a number of such substances are listed with their standard free energies of hydrolysis.

Relationship Between Free Energy and Oxidation It is now known that 60 to 80 per cent of the total free energy available in foodstuffs is released during the oxidative phase of metabolism.² Oxidation may be defined as the loss of electrons and reduction as the gain of electrons. A typical oxidation-reduction system may be written as



where n is the number of electrons (e) involved in the reaction. The maximum available work that can be obtained from such a reaction in which a transfer of electrons occurs may be described in terms of the potential difference or electromotive force. All oxidation-reduction reactions of which there are many in nature may be so described. The equation relating ΔF° to the electromotive force is

$$\Delta F^\circ = -nFE$$

where n is the number of electrons taking part in the reaction, F is the faraday (23,068 calories per volt equivalent) and E is the potential or experimentally measured quantity. E is related to

TABLE I

STANDARD FREE ENERGIES OF HYDROLYSIS OF SOME IMPORTANT PHOSPHATE COMPOUNDS

Compound	Bond Type	ΔF Cal
α Glycerophosphate	ester phosphate	-9200
Glucose 6 phosphate	ester phosphate	-3000
Fructose 6 phosphate	ester phosphate	3000
Adenosine triphosphate	pyrophosphate	-12 000
Inorganic pyrophosphate	pyrophosphate	-1 000
Creatine phosphate	nitrogen phosphate	-11,800
Phosphorylenolpyruvate	enol phosphate	-1 900
Acetylphosphate	acyl phosphate	-15 000
Acetyl coenzyme A	thiol phosphate	-13 000

concentration of the oxidized and reduced forms by the following relation

$$E = E^{\circ} + 2.303 \frac{RT}{nF} \log \frac{(\text{oxid})}{(\text{red})}$$

where E is the standard electrode potential and equal to E° when the system is half reduced

Work of the Heart The mechanical work performed by the heart during each contraction has customarily been calculated from the sum of the pressure energy and kinetic energy imparted to the volume of blood ejected. The differences between pressures in aorta and pulmonary artery makes it necessary to perform calculations for the left and right ventricles individually. The work of atria is usually neglected. The work for each ventricle is expressed by

$$W = qP + \frac{\rho qV^2}{2G}$$

where q is the volume of blood ejected, P the mean arterial pressure, V the velocity of ejection and ρ the density of blood. G is the gravitational constant.

For the left ventricle $q = 60 \text{ cm}^3$ $P = 100 \text{ mm Hg} = 136 \text{ cm water}$ $V = 50 \text{ cm/sec}$ and $\rho = 1.06$ in the human at rest then

$$W_1 = 60 \times 136 + \frac{1.06 \times 60 \times 50}{2 \times 980}$$

$$8160 + 80 = 8240 \text{ g-cm}$$

For the right ventricle $q = 60 \text{ cm}^3$ $P = 20 \text{ mm Hg} = 27 \text{ cm water}$ $V = 50 \text{ cm/sec}$ then

$$W = 1620 + 80 = 1700 \text{ g-cm}$$

The total work under resting conditions $= W_1 + W = 9940 \text{ g-cm}$ or approximately 100 g m per beat. For a heart rate of $70/\text{min}$ this amounts to 7000 g m/min or 420 kg m/hr . In hard exercise the total work per hour may reach 4000 kg m/hr .

In heat equivalents this amounts to 983.9 cal/hr during rest and 11370.8 cal/hr during hard exercise.

The Efficiency of the Heart The overall efficiency of the heart can be pictured as comprising the purely mechanical efficiency as a pump and the efficiency of conversion of chemical energy derived from the fuel supplied into external mechanical work done on the blood circulation.

The efficiency of conversion of chemical energy into mechanical work by the muscle fibers can only conveniently be estimated by comparing the chemical energy released and the external work developed over a given period. It is usually calculated indirectly by comparing the work performed with the myocardial usage of O_2 . In the past calculations were made neglecting the contribution of anaerobic metabolism to energy production and O_2 consumption of the resting non-beating heart. There is no evidence to indicate that anaerobic metabolism contributes much to energy production. On the other hand recent studies have shown that the O_2 consumption of the non-beating heart is 20 to 35 per cent of that of the beating heart.⁴ In the case of the dog heart Bing has shown that the O_2 usage of the beating heart is 25.11 ml/min and 4.83 ml/min for the arrested heart. The O_2 utilization of the latter is 20 per cent of that of the former. Therefore 20 per cent of the total O_2 usage

of the left ventricle is used for basal metabolic processes exclusive of work. The efficiency during contraction is

$$\frac{\text{work of heart}}{\text{O}_2 \text{ usage/min} \times 2.059 \times 0.8} \times 100$$

where 2.059 is the energy equivalent of 1 ml of O₂ at an R.Q. of 0.82 and 0.8 is the fraction used in contractile work. Making the suggested corrections Bing calculates myocardial efficiency to be 37 per cent in the dog and 39 per cent in man. He further estimates that 87 per cent of the energy stored in ATP is converted into mechanical work by the heart.

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Chapter III

CHEMISTRY OF THE NORMAL HEART

PART I FREE ENERGY RELEASE

Introduction The heart is a compact muscle mass with the fundamental function of converting chemical potential energy into mechanical work. As in skeletal muscle heart derives its energy from the breakdown of certain essential foodstuffs by a complex series of enzyme catalyzed reactions. The purpose of these reactions is to release free energy in such form that it can be stored in energy rich compounds. ATP is the prototype. The free energy is eventually utilized by the contractile apparatus to perform mechanical work.

There is every reason to believe that the fundamental process connected with energy production is the oxidation of foodstuffs. The essential substrates are carbohydrate in the form of glucose and fatty acid. Oxidation of foodstuffs is a step wise process which being diverse for the various substances initially is quite uniform as the substrates reach the final stages of free energy release. The reactions proceed simultaneously under the influence of enzymes. The activity of these enzymes in turn is dependent upon smaller organic substances called *coenzymes*. Coenzymes are in large part derivatives of the well known vitamins of the B complex and constitute a means by which the nutritional state may influence metabolism.

Hormones are another important class of chemical regulators. They appear to exert their effects on metabolism by controlling the transport of essential metabolites across biological membranes. For example it has been shown that insulin enhances the entry of glucose into the cell independent of any effect on metabolic reactions.¹

In the present chapter we shall briefly outline what is known of the intermediate steps involved in free energy release in the normal heart. Much of what is to be said concerns free energy metabolism as we know it in skeletal muscle. Recent studies strongly indicate that both heart and skeletal muscle derive their energy for contraction in the same manner and from the same foodstuffs. Information has been obtained necessarily from studies on tissue slices, homogenates and isolated enzymes. However, investigations on the intact heart both *in vivo* and *in vitro* leave no doubt that metabolic patterns to be discussed are of physiological significance.¹

As pointed out earlier, the prime function of metabolism is the release and subsequent trapping of free energy in the form of energy rich compounds for eventual utilization by the contractile apparatus in the performance of work. There is strong evidence that one of the primary sources of free energy is carbohydrate in the form of glucose. The ultimate breakdown of glucose can be classified into several types of transformations: glycolysis, Krebs' citric acid cycle, and the electron transport system.

Reactions of Glycolysis. Glycolysis can be defined as the sum total of enzyme catalyzed reactions in which glucose is degraded to two molecules of lactic acid. In order for glucose to enter this pathway it must be phosphorylated to glucose 6 phosphate by adenosinetriphosphate and the enzyme hexokinase. Once formed, glucose 6 phosphate may be incorporated into glycogen by way of glucose 1 phosphate, or it can enter the main glycolytic series of reactions ultimately to be broken down into two molecules of pyruvate or lactate. Under anaerobic conditions, pyruvate is converted to lactate. In Figure 1 we have outlined some of the major reactions involved.

Even though this is not the proper place to discuss every step of glycolysis in great detail, certain of the steps are very important to our understanding of free energy release. These reactions illustrate in a most elementary way how chemical potential energy is released and conserved in the form of high energy compounds.

(1) **Triose Phosphate Dehydrogenase Phosphorylglycerol Kinase Reaction.** The oxidation of 3-phosphoryl-D-glyceraldehyde formed by the cleavage of fructose 1,6-diphosphate is an extremely

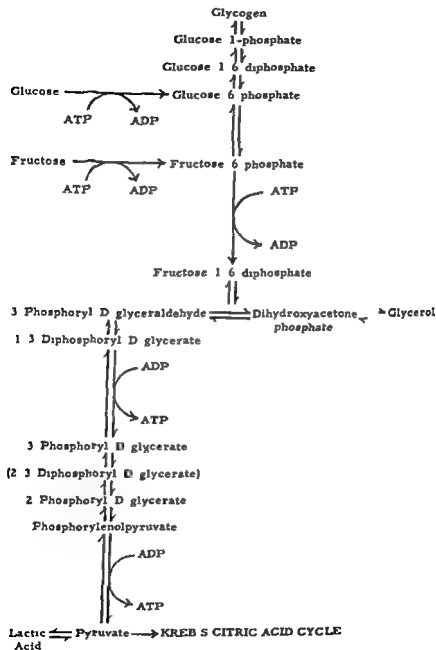
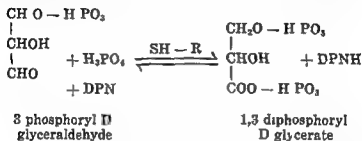


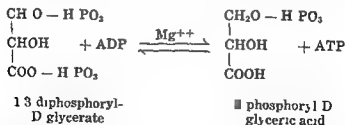
Figure 1 Outline of the glycolysis reaction

important reaction. It illustrates in a clear manner those chemical events associated with rearrangement of bond energy of the original substrate glucose whereby it is transferred and conserved in energy rich compounds. The nature of this reaction was established in Warburg's laboratory.⁴



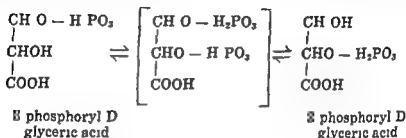
DPN is the oxidized form of diphosphopyridine nucleotide a derivative of the B vitamin nicotinamide. The mammalian enzyme contains DPN in an enzymatically active form. One mole of the enzyme contains two moles of firmly bound DPN. Cysteine or another SH compound is required for full activity.⁶ At the present time there is good evidence that essential SH groups are involved in the binding of DPN. The product of the reaction 1,3 diphosphoryl D glycerate contains an acyl phosphate bond a high energy phosphate bond (see Table I Chapter II).

The acyl or carboxyl phosphate of 1,3 diphosphoryl D glycerate is transferred to ADP by the enzyme phosphorylglycerol kinase

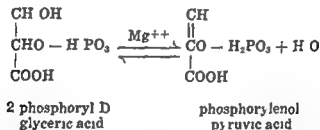


This illustrates how free energy is conserved. The enzyme has been crystallized from yeast by Bucher and appears to be specific for all components of the reaction. Like other kinases this enzyme requires magnesium ions.

(2) **Phosphorylglyceryl Mutase Enolase Reactions** By the action of these two enzymes 3 phosphoryl D-glyceric acid is converted to the phosphate ester of enol pyruvate phosphorylenolpyruvate by the following reactions

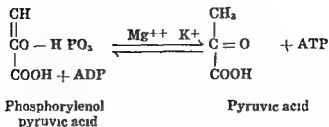


Enolase dehydrates 2 phosphoryl D glyceric acid to phosphorylenolpyruvic acid



Both phosphorylglyceryl mutase⁸ and enolase⁹ have been crystallized. Enolase requires magnesium ions and is inhibited by fluoride which forms a Mg F PO_4 complex that competes with magnesium ions¹⁰

(3) **Pyruvate Kinase** The product of the above sequence of reactions phosphorylenolpyruvic acid contains an enol phosphate bond. The phosphate group is transferred to ADP by pyruvate kinase. The equilibrium is greatly in the direction of ATP formation.



The enzyme has been crystallized from human muscle¹¹ and requires potassium ions in addition to magnesium ions. It is quite non specific in its nucleotide requirement in that the diphosphates of adenosine, uridine, cytidine and guanosine can all accept phosphate from phosphorylenolpyruvic acid.¹

Another important feature of the glycolysis reaction is the key role played by phosphate and phosphate acceptors such as ADP. In glycolysis the metabolism of glucose is tightly coupled with that of phosphate. In order for the several reactions to occur phosphate is required as well as phosphate acceptors. If the level of ADP falls the overall rate of glycolysis decreases. A sudden increase in the level of ADP, as might occur following vigorous muscle activity where large amounts of ATP are hydrolyzed to PO_4 and ADP, could increase the overall rate of glycolysis. Such a mechanism might serve to explain how muscle function controls the rate of release of free energy, or why after vigorous muscular activity the metabolism of carbohydrates increases.

Kreb's Citric Acid Cycle and the Electron Transport System

Since the heart is essentially an aerobic organ¹² richly endowed with respiratory enzymes, pyruvate is transformed rather rapidly by pyruvic oxidase in the presence of certain coenzymes (thiamine pyrophosphate and lipoic acid) to acetyl coenzyme A.¹⁴ Acetyl coenzyme A is a derivative of pantothenic acid and has the following structure.¹⁵

In the presence of a condensing enzyme the two carbon fragment of acetyl coenzyme A is attached to oxalacetate to form citrate, thereby initiating a cyclic process of reactions known as Kreb's citric acid cycle.¹⁶ The net result of this cyclic process is the release of two molecules of CO_2 and 8 hydrogen atoms by enzymatic

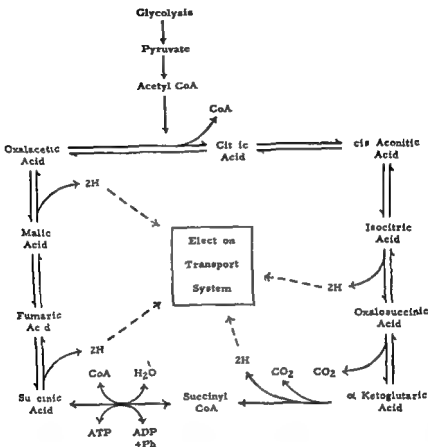


Figure 1 Outline of Krebs citric acid cycle

Belitser¹⁵ showed that the ΔF for the transfer of a pair of electrons from the substrate level in the citric acid cycle to molecular oxygen was approximately $-55,000$ cal/mole of oxygen. Since the formation of 1 mole of ATP requires an input of $12,000$ cal, it is evident that approximately 4 moles of ATP may be generated in the transport of each pair of electrons to oxygen providing suitable coupling mechanisms exist.

Calculations¹⁵ reveal that approximately 80% of the total free energy obtained from the degradation of glucose is conserved by

OXIDATIVE PHOSPHORYLATION

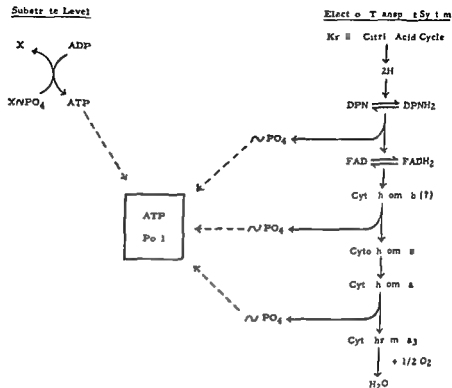


Figure 3 Outline of the electron transport system

the process of oxidative phosphorylation. The remaining free energy is trapped by phosphorylation at the substrate level. The detailed mechanism of the oxidative phosphorylation reaction is not known with certainty at the present time. This is probably due to the fact that it has been extremely difficult to isolate the individual enzymes in a high state of purity.

Energetic Considerations An analysis of the energetics of the following reaction



indicates that the free energy change involved in the conversion of 1 mole of glucose to CO_2 and H_2O is approximately $-688,000$ cal.

If one $\sim \text{PO}_4$ represents a $\Delta F_{\text{hyd}}^{\text{hyd}}$ of $-12\,000$ cal we have $688\,000/12\,000$ or approximately $57 \sim \text{PO}_4$ theoretically available from the complete oxidation of glucose

When glucose is degraded to pyruvic acid some $50\,000$ cal of free energy are theoretically available ¹ An analysis of Figure 1 will show that in the anaerobic breakdown of glucose to pyruvate a total of four ATP molecules are synthesized However of these four one is utilized in the phosphorylation of glucose and one in the conversion of fructose 6 phosphate to fructose 1,6 diphosphate Hence a net synthesis of only 2 ATP molecules occurs Therefore the efficiency is

$$\frac{2 \times 12\,000 \times 100}{50\,000} = 48\%$$

In the complete oxidation of pyruvate approximately $630\,000$ cal of free energy are made available Accompanying this process approximately 38 molecules of ATP are synthesized ² Therefore the efficiency of oxidative phosphorylation would be

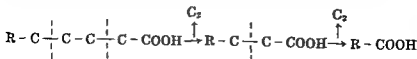
$$\frac{38 \times 12\,000 \times 100}{630\,000} = 72\%$$

and the overall efficiency would be

$$\frac{(38 + 2)(12\,000)(100)}{688\,000} = 70\%$$

Alternative Sources of Energy Recent studies indicate that when the concentration of glucose in the blood falls below a certain level the myocardium has the capacity to utilize fatty and amino acids for free energy sources ³ In fact there is some evidence that the major free energy source for the heart may well be fatty acids ⁴ Fatty acid metabolism is of considerable interest since mole for mole the complete degradation of a fatty acid makes available considerably more free energy than glucose breakdown

(1) **Fatty Acid Metabolism** Knoop ⁵ first showed that fatty acids were degraded by β oxidation from the carboxyl end of the molecule in the following manner



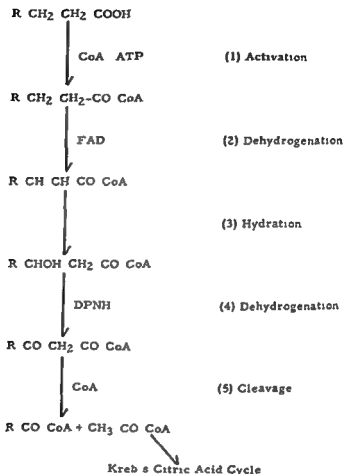
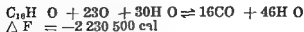


Figure 4 - Outline of fatty acid degradation

It is now known that in animal tissues all the enzymes necessary for the activation and degradation of free fatty acids exist. A typical sequence of reactions is presented in Figure 4. It can be seen from the figure that in the β oxidation of 1 mole of fatty acid only 1 mole of ATP is utilized regardless of the chain length. Once the fatty acid is in the form of the acyl coenzyme A derivative the thiol ester group is preserved until it condenses with oxalacetate to enter the citric acid cycle for further metabolism.

The total free energy release accompanying the complete

degradation of a fatty acid such as palmitic acid has been determined. The reaction is as follows



The release of free energy occurs in two major steps ⁸

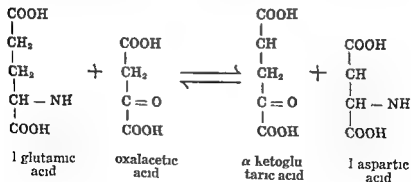
- (1) palmitic acid \rightleftharpoons 8 acetyl coenzyme A + 34ATP
- (2) 8 acetyl coenzyme A + 16H⁺ + 16O \rightleftharpoons 16CO₂ + 24H₂O + 104ATP

Thus a total of 138 molecules of ATP are synthesized per mole of this fatty acid metabolized and the efficiency for the process is

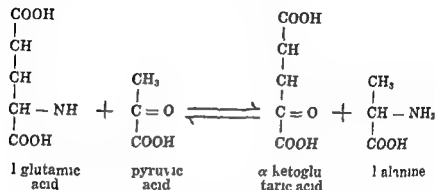
$$\frac{(138/1) \cdot 12000 \times 100}{2230500} = 71\%$$

(2) *Amino Acid Metabolism* : The reversible transfer of an amino group from an amino acid to a keto acid was first described by Braunstein and Krisman ⁹. This reaction is catalyzed by a group of enzymes called transaminases. These enzymes require derivatives of pyridoxal phosphate as coenzymes. Two of these enzymes are of considerable interest and will be discussed briefly as they illustrate how certain amino acids enter the Krebs citric acid cycle.

(1) Glutamate aspartate transaminase. This enzyme catalyzes the following reaction



(2) Glutamate alanate transaminase This enzyme catalyzes the following reaction



The highest concentration of glutamate aspartate transaminase is found in the myocardium and liver³⁰ Apparently in severe liver or myocardial disease this enzyme is released into the serum and serum levels of this enzyme are thought to measure the extent of damage³¹

Summary In the above considerations we have seen that the release of free energy by the combustion of foodstuffs in the myocardium occurs in two major phases. In the first phase small molecules such as glucose, fatty and amino acids are incompletely metabolized to one of three constituents: acetyl coenzyme A, α ketoglutarate or oxalacetate. Acetyl coenzyme A constitutes the greater amount. Two thirds of the carbon atoms of carbohydrates, all the carbon atoms of the common fatty acids and approximately one half of the carbon atoms of amino acids yield acetyl coenzyme A.³

The three end products of the first phase are metabolically similar. They take part in the second major phase of free energy release: the Krebs citric acid cycle and the electron transport system. The latter systems may be considered as the final common pathway for the complete combustion of all foodstuffs. In Figure 5³¹ we have attempted to summarize in a diagrammatic form the major metabolic events associated with free energy release and its subsequent trapping in the form of ATP.

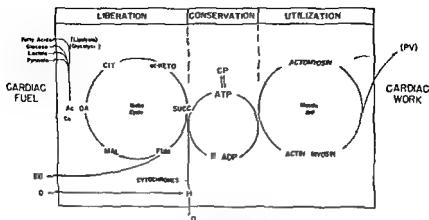


Figure 5 Schema of free energy release, conservation, and utilization in cardiac muscle. Reproduced by permission Olson and Piatnek. *Ann N Y Acad Sci* 1959

Localization of Enzymes in the Cell: There are a number of factors that influence the rate of enzymic reactions within the cell. These include temperature, pH, coenzymes, redox potentials, concentration of reactants, and the presence or absence of inhibitors. Another extremely important factor is the intracellular organization of enzymes. Organization facilitates a rapid utilization of the products of one enzymatic reaction by another. The complex series of reactions discussed in the present chapter require participation of many enzymes whose activities are *interrelated and coordinated*. The formation of pyruvate from glucose requires the coordinated and sequential action of at least ten enzymes. As a result of this action, a *portion of the chemical potential energy of glucose is conserved*.

Although we are now fully aware that many cell enzymes as well as *enzyme systems* function quite well in simple solution, the living cell cannot be considered to be simply a bag of enzymes. Histology and electron microscopy tell us that the living cell has a *beautifully elaborate structure*. Classically the cell contents have been divided into cell membrane, cytoplasm, and nucleus. The cell cytoplasm is a heterogeneous system containing many particulate

structures such as mitochondria microsomes glycogen granules myofilaments and endoplasmic reticulum³⁴

Due to the development of the technique of homogenization and differential centrifugation by Bensley and Hoerr³ Claude³⁵ and Hogeboom³ we have been able to establish considerable information concerning the distribution and localization of enzymes within the cell

(1) *Nucleus* It was earlier thought that a number of enzymes were located within the nucleus or on its limiting membrane. However, modern isolation and purification techniques have established that the nucleus is primarily the center of nucleotide metabolism and that all enzyme systems involved in free energy release are absent

If the nucleus does play a role in free energy release it is probably an indirect one. There is good evidence that nuclear desoxyribonucleic acid and ribonucleic acid control the synthesis of the cytoplasmic enzymes involved in the release of free energy.

The studies of Mazia³⁶ indicate that the glycolytic and respiratory enzyme systems remain intact for long periods after enucleation.

(2) *Mitochondria* Mitochondria consume oxygen and contain all of the enzymes necessary to catalyze the reactions in the Krebs citric acid cycle fatty acid oxidation electron transport system and oxidative phosphorylation³⁷. The major role of these organelles in aerobic metabolism and ATP production have established them as the power houses of the cell. Irreversible damage to isolated mitochondria occurs after periods of ageing inoxia and exposure to certain surface active agents. ATPase activity usually increases under these conditions and the esterification of inorganic phosphate into high energy phosphite compounds is diminished.

Low concentrations of certain chemical agents such as 2,4 dinitrophenol and congeners⁴⁰ are also known to uncouple oxidative phosphorylation in mitochondria almost completely while actually stimulating (up to 100%) or having little effect on respiration. These agents appear to stimulate an ATPase or phosphatase like activity⁴¹. The addition of ATP partially reverses their action. Other uncouplers include azide dicoumarol⁴² and thyroxine⁴⁴.



Figure 6a An electron micrograph of rat ventricle $\times 50,200$. Reproduced by permission Siekevitz *Ann N Y Acad Sci* 1959

None of these chemical agents interfere with phosphorylation at the substrate level as occurs during glycolysis

Many authors have presented evidence that mitochondria may function in cellular differentiation⁴ and cell membrane transport⁴⁶

In Figure 6a we have presented an electron micrograph of typical mitochondria⁴⁷ from heart muscle (sarcosomes). A diagram

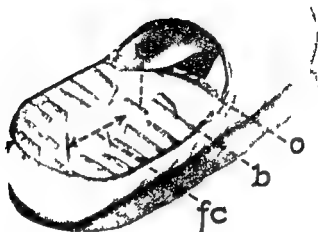


Figure 6b A tri dimensional model of a mitochondrion c crista b branched cristae fc intramitochondrial space o limiting double walled membrane Reproduced by permission *J Histochem Cytochem* 11:85 1963 (Palade)

matic sketch is shown in Figure 6b¹⁰ The typical mitochondrion has been shown by a number of investigators Claude and Fullam¹¹ Dalton¹² Palade¹³ and Sjostrand and Rhodin¹⁴ to possess a double walled limiting membrane approximately 80 Å thick. Invaginations continuous with the inner of the two membranes partially occupy the intramitochondrial space. These have been called cristae mitochondriales by Palade. The dimensions of mitochondria from heart muscle range from 0.3 to 1.7 μ in length and 0.2 to 1.0 μ in diameter.

It is well known that these particulates have certain osmotic or colloidal properties in that they swell and shrink reversibly in various media.¹⁵ Permeability studies indicate a more or less functional membrane. The dense inner matrix of folded cristae present a very large surface area for adsorption of substrates and metabolic reactions.

The exact localization of the various enzymes in the mitochondrion is not established. Upon fragmentation (by sonic vibration or surface active agents) insoluble submitochondrial units have been shown to possess the ability to transport electrons or to mediate oxidative phosphorylation⁵⁴. Most studies indicate that the citric acid cycle and fatty acid oxidation enzymes are in a more soluble state within the intramitochondrial matrix.⁵

(3) **Microsomes** Microsomes are much smaller particles than mitochondria and sediment in higher gravitational fields.⁵ Microsomes probably form a part of the fine tubular network in the cytoplasm called the endoplasmic reticulum.⁵⁸ These structures contain large amounts of lipids and ribonucleic acids.⁵ Studies on the incorporation of amino acids suggest that protein synthesis occurs here.^{59,60}

(4) **Soluble Enzymes** Many of the intracellular enzymes appear to be present in simple solution in the cytoplasm. Enzymes involved in the glycolysis reaction are found in the cytoplasmic fraction.⁶⁰ However, for full activity of this multi-enzyme system the mitochondrial and microsomal fractions are required.⁶¹ Many of the enzymes catalyzing ATP requiring reactions are also in the soluble fraction as well as those catalyzing the urea cycle.⁷

(5) **Cell Membrane** Two enzymes of great physiological importance are present in the cell membrane at least in the case of red cells and squid axon: cholinesterase⁶² which hydrolyzes acetylcholine and an ATPase.^{64,6} There is strong evidence that cholinesterase controls the rate of passive transport of cations in excitable tissue⁶³ while ATPase is involved in active transport of these electrolytes.⁶⁷

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Chapter IV

CHEMISTRY OF THE NORMAL HEART

PART II FREE ENERGY UTILIZATION

Introduction In the preceding chapter we have discussed some of the chemical mechanisms involved in free energy release from essential foodstuffs. In the present chapter we intend to review the known physico chemical events associated with the utilization of free energy for the performance of work. There is now good evidence that the free energy utilizing system in the myocardium is the protein complex actomyosin. ATP serves as its major energy source. A mere knowledge of the physico chemical properties of actomyosin and its interaction with ATP is not sufficient to describe completely the events accompanying muscle contraction. A coherent and integrated visualization of the events in contraction requires additional information necessary to answer the following questions:

What is the nature of the stimulus (excitation-contraction coupling)? What is the chemical and physical nature of the components actually participating in contraction? What changes occur between the participating components during the shortening process? What is the nature of the physico chemical events whereby the free energy of hydrolysis of ATP is utilized in the performance of mechanical work? The first two questions may be answered with some degree of certainty.

Numerous models have been proposed to explain the contraction process but the subject remains highly speculative. This is only natural in view of the wide variety of techniques and procedures employed by investigators with diverse backgrounds. Each has his own viewpoint. It should be pointed out at this time that the authors have little confidence in chemical theories which make

no attempt to account for morphological or physiological events accompanying the contractile response

As was mentioned previously the information in the present chapter in large part concerns skeletal muscle because most of our knowledge has been obtained from studies on this tissue. However there is little reason to doubt that cardiac muscle is similar to skeletal muscle. There are differences in morphology and chemistry but these are of a quantitative and not of a qualitative nature.

Morphological Considerations The histological structure of both striated and cardiac muscle as well as changes that occur during the contractile response were well described by 1900.¹ With the rapid advances in muscle chemistry the contributions of the 18th century microscopists were soon forgotten. The development of the techniques of electron microscopy, x-ray diffraction, optical rotation and others has stimulated reinvestigation of muscle structure and activity.

The unit of structure in both striated and cardiac muscle is the muscle fiber which measures 10 to 100 μ in diameter and whose length varies considerably (a few millimeters to several centimeters). In cardiac muscle there is considerable branching and anastomosing of muscle fibers giving the appearance of a syncytium (Figure 7). Each branch of the cardiac muscle fiber terminates at an intercalated disc which according to recent electron microscope studies represents a portion of the cell border or membrane.² The intercalated discs are actually continuations of the inner and functional layer (plasma membrane) of the cell membrane (sarcolemma). Each disc measures 130 to 150 μ wide. It is composed of two opposing thickened segments of the plasma membranes of two cells separated by a narrow interspace.

The muscle fiber contains bundles of myofibrils the contractile units of the cell. The myofibril measures 0.5 to 2.0 μ in diameter.³ Electron micrographs show that there is no boundary membrane separating the myofibril from the various substances in the sarcoplasm.⁴ A free access of substrates, coenzymes and ions is entirely probable.

In Figures 8a and b typical electron micrographs of striated⁵



Figure 7 An electron micrograph of rabbit myocardium $\times 4800$ D intercalated disc N nucleus Z Krause's membrane (Z line) Reproduced by permission *J Physiol Biochem Cytol* 1957 (Muir)

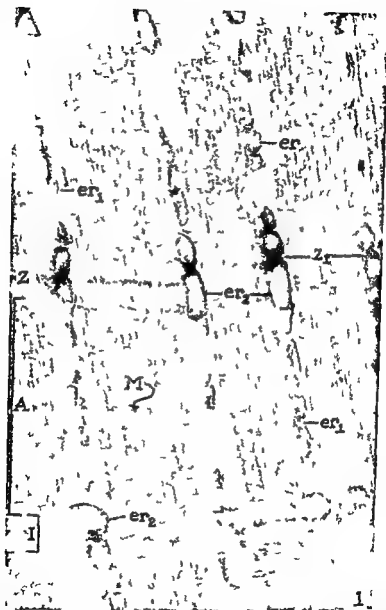


Figure 81. An electron micrograph of striated muscle (crutch myotome *Amblystoma* larva) 48,000. er endoplasmic reticulum / Kruse's membrane (Z line) M M line in H zone I I band A A band. Reproduced by permission J Biol Phys Biochem Cytol 1951 (Porter and Falick)



Figure 8b An electron micrograph of rat ventricle $\times 15,500$ er endoplasmic reticulum A A band I I band II II zone Z Krause's membrane (Z line) L lipid body Note large number of sarcosomes (mitochondria) between myofibrils Reproduced by permission *J Biophys Biochem Cytol* 1957 (Moore and Ruska)

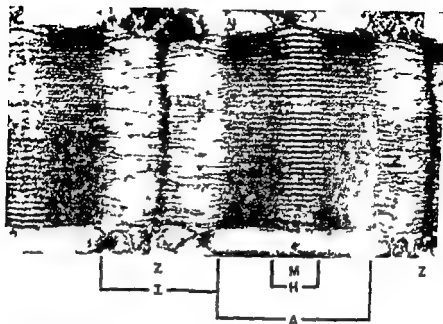


Figure 11 An electron micrograph of an isolated myofibril $\times 50,000$. Reproduced by permission *J Biophys Biochem Cytol* 1967 (Huxley)

and cardiac muscle³ are presented. They illustrate the essential structural features as well as the differences which exist in the two tissues. Figure 9⁶ is an electron micrograph of an isolated myofibril.

The cross striations along the long axis of the fibril are due to differences in optical and staining properties. These striations give the appearance of a series of discs or bands lined up side by side. The darker or anisotropic regions are referred to as A bands; the lighter or isotropic regions are referred to as I bands. In the center of the I band there appears a thin highly refractile zone (Krause's membrane)⁷ called the Z line.

Z lines of adjacent myofibrils are interconnected by continuous membranes.⁸ They appear to divide the whole muscle fiber transversely but in an ascending and descending step like fashion. They eventually merge with the plasma membrane (inner layer of the sarcolemma) at the outer border of each fiber.

In the middle of the A band there is a less dense region called the H zone which is particularly evident in stretched fibers. In the center of the H zone another thin region of somewhat greater density is observed. The latter is referred to as the M line. There is some evidence that adjacent M lines are continuous similar to the Z lines.⁸

The sarcomere is the longitudinal unit of the myofibril and is delineated by two Z lines. Therefore it includes one half of an I band at either side of an A band. In heart it is 1.1 to 2.4 μ long.¹⁰

On closer examination of electron micrographs one can see that each myofibril is composed of a number of myofilaments arranged in parallel (see Figure 9). These are basically of two types: a thin, tenuous filament and a much denser one. The thin filaments begin at the Z lines, extend through the I band into the A band, and end in the H zone. In the A band the thin I band filaments interdigitate with the thicker A band filaments.

In a cross section (Figure 10)¹¹ through the A band, it can be seen that the thin filaments (diameter 40 Å) are located in a regular

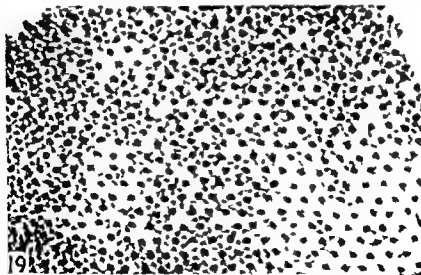


Figure 10 : An electron micrograph cross section through A band region $\times 150,000$. Reproduced by permission *J Biophys Biochem Cytol* 1957 (Huxley)

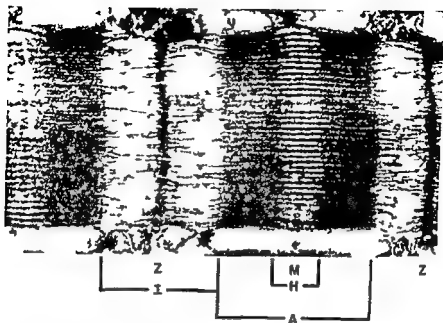


Figure 9 An electron micrograph of an isolated myofibril $\times 50,000$. Reproduced by permission *J Biophys Biochem Cytol* 1957 (Huxley)

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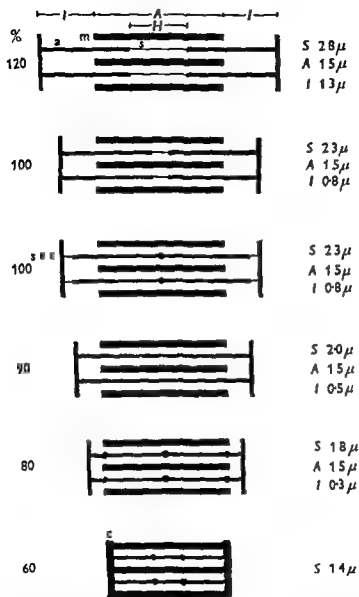


Figure 11 Diagrammatic longitudinal sections of sarcomeres during stretch and contraction ■ sarcomere width A A band width I I band width s elastic component in H zone sec series elastic component near Z line m thick A band filament a thin I band filament ■ contraction band Reproduced by permission *Symposia Soc Exp Biol* 1955 (Hanson and Huxley)

These findings led the Huxleys to propose the sliding model of contraction to be discussed later in the chapter

Chemical Considerations : Almost a century ago Kuhne extracted a protein from muscle by treating the tissue with strong salt solutions¹⁴. He gave the name myosin to his preparation. For the next one hundred years the name myosin was used to denote the substance or substances which could be extracted from muscle by solutions of relatively high ionic strength. In 1930 von Muralt and Edsall¹⁵ showed that these solutions exhibited a strong double refraction of flow suggesting the presence of elongated and rather large molecules.

During the late thirties and early forties a number of investigators^{16, 17} showed that ATP had profound effects on the physico-chemical properties of myosin. On the addition of ATP to myosin solutions the double refraction disappeared and the viscosity decreased. Furthermore threads prepared by squirting myosin solutions into water contracted in the presence of ATP. In 1939 Engelhardt and Ljubimov¹⁸ showed that myosin contained ATPase which split off the terminal phosphate of ATP forming free phosphate and ADP. Thus it was only natural to assume that these reactions might be the chemical basis of muscle contraction.

In the early forties it was discovered that classical myosin was in reality a mixture of two proteins myosin and actin¹⁹. Actin exists in two forms: globular or G actin and fibrous or F actin. F actin interacts with myosin to form a new protein actomyosin. Thus the myosin of Kuhne can be considered to be actomyosin.

From the many sided evidence accumulated in the last fifteen years these discoveries encompass all that seem basically essential in the chemistry of muscle contraction: that is myosin acting as an ATPase, actin and ATP.

(1) **Myosin**^{19, 20} : The protein myosin can be extracted from muscle with about half molar salt solution at pH 6.5. It has a molecular weight of the order of 400 000 to 450 000. Myosin is an elongated particle being approximately 1600 Å long and 50 Å wide. Well purified myosin splits the terminal phosphate group of ATP

and related nucleotides¹ Myosin ATPase is activated by calcium ions and inhibited by magnesium ions By the action of trypsin each myosin molecule can be degraded into one heavy fragment (H meromyosin) and two light fragments (L meromyosin)² The heavy fragment possesses all of the ATPase activity

There have been only a limited number of studies on the physico-chemical nature of heart contractile proteins According to Gergely³ myosin obtained from heart muscle has essentially the same molecular weight and shape as that of skeletal muscle and its reactions with ATP are similar However Ellenbogen and collaborators³ present values for the molecular weight of myosin which are considerably less (223 000) The ATPase activity of heart myosin is about one third that of skeletal muscle myosin and it appears more resistant to trypsin and chymotrypsin degradation

(2) *Actin*²⁰⁻²² Actin is an unusual protein in that it exists in two forms a globular and a fibrous form²⁴ G actin has a molecular weight of approximately 70 000 and contains approximately one mole of ATP per mole of G actin G actin readily polymerizes to form F actin Only the latter is capable of specific interaction with myosin Upon interaction ATP is split into ADP and inorganic phosphate

(3) *Tropomyosin* Tropomyosin comprises approximately 4% of the myofibrillar protein and it has been crystallized by Bailey²⁵ At low salt concentrations tropomyosin exists as a highly viscous thread in solution it is largely depolymerized at 0.16 M KCl (intracellular concentration) and has a particle weight of 120 000 Actin and tropomyosin combined have a similar amino acid composition to myosin in all species tested²⁶ Tropomyosin has been interpreted as a precursor for some subunit of myosin as an elastic component of the myofibril and as the substance in cross bridges²⁷

(4) *Delta Protein* Delta protein²⁸⁻³⁰ can form a complex with myosin and cause dissociation of actomyosin It is believed to be a derivative of actin and may serve as the underlying structural component which facilitates proper orientation of actin myosin and tropomyosin in the myofibril It may also represent

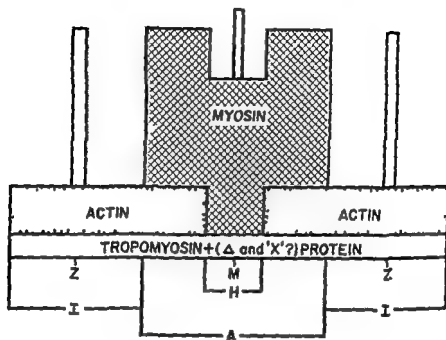


Figure 12 General distribution of the myofibrillar proteins in the sarcomere

in elastic component (5 filaments)¹⁴ in the H zone. At least part of the 15% of myofibrillar protein which remains after extraction of myosin, actin and tropomyosin is believed to consist of delta protein.

Figure 12 diagrammatically illustrates the general distribution of the myofibrillar proteins in the sarcomere.

Interaction of Actomyosin and ATP¹⁵ Actomyosin is formed readily by the reaction of F actin and myosin. The viscosity of actomyosin solution is much higher than the sum of the viscosities of the two individual components. Actomyosin ATPase activity is activated by both magnesium ions and calcium ions. In the presence of magnesium ions its activity far exceeds that of myosin.

Striking physico-chemical changes are induced when ATP is added to solutions of actomyosin or to actomyosin threads. The double refraction of flow disappears and the viscosity of the solutions decreases markedly. ATP causes contraction of the threads.

These changes have been interpreted as resulting from the action of ATP to dissociate actomyosin into actin and myosin

Relaxing Factor The existence of a sarcoplasmic component in muscle which could induce relaxation in isolated systems was first shown by Marsh³¹ and Bendall.³² They and other workers have tried to identify this component with various phosphate transfer ring enzyme systems such as myokinase, creatine phosphokinase and phosphoenol pyruvate pyruvic kinase. All of these transfer phosphate to ADP and their relaxing effect is activated by Mg ions and inhibited by Ca ions. The fact that muscle ATPase activity is depressed concurrently with the relaxing effect suggests a direct correlation of the former with tension development.

Recent evidence indicates that relaxation depends on a critical level of ATP in the center of the muscle fiber which could be effected by any of the enzyme systems mentioned. However in single muscle fiber preparations these systems do not always cause relaxation and may increase tension if the ATP concentration is low enough. It is now believed that the relaxing system consists of at least two components: a particulate component and a dialyzable labile substance which is readily absorbed on charcoal. Neither of these is one of the transphosphorylating systems mentioned. Pyrophosphate may replace the dialyzable factor but it is not identical with it.

The answers to the important questions of whether such systems are operable in intact muscle and especially in the myocardium are only speculative.

Localization of Contractile Proteins in Muscle In recent years with improvement in physical and chemical techniques there have been a number of attempts to relate the molecular components of muscle with the ultrastructure of the cell and the actual process of contraction. The most elaborate of these studies have been those of H. E. Huxley,^{6, 11, 13, 15} A. F. Huxley,^{1, 24} and collaborators. By the use of optical and electron microscopy, x-ray diffraction techniques and specific chemical methods for extracting various proteins from isolated muscle fibers they reached the following conclusions about the distribution of contractile proteins

Striated muscle (including cardiac) is built up of two overlapping interdigitating myofilaments. One is a set of thin filaments extending in either direction from the Z line to the boundary of the H zones and the other is a thicker set extending from one end of the A band to the other. When muscle fibrils are treated with solutions that are known to remove myosin 60 to 70% of the protein material is selectively removed from the A band. Approximately 70% of this protein is actually myosin; the remainder is γ protein.³⁵ The material or myofilaments left are the thin filaments which extend from the Z membrane to the former border of the H zone. When the treated myofilaments are examined with the electron microscope the thick filaments which formed the A band are missing and only the thin filaments remain.

When the myosin extracted preparations are treated with solutions that remove actin an additional 15% of the original myofibrillar protein is removed. The fibrils lose all their visible material between the Z lines and the H zones. The Z lines and some substance (delta protein?) which maintains structural integrity remain.

Thus a remarkably simple picture emerges of two overlapping sets of filaments built primarily from two structural proteins: actin and myosin. The thin filaments are composed of actin and the thick dense filaments are composed of myosin. Neither extends through the entire length of the sarcomere. The thin filaments are present both in the I and part of the A bands; the thick filaments are in the A band only. The two interdigitate in the A band. Such a system gives the banded appearance to the muscle fibril.

A number of recent studies suggest that the A band contains ATP and many of the enzymes essential for oxidative phosphorylation independent of muscle mitochondria.³⁶

Theories and Models of Muscle Contraction At the present time there are two basic theories that attempt to correlate the molecular and physiological events accompanying the contractile response. The first may be called the one filament hypothesis or the flexible polyelectrolyte theory. The second may be called the two filament hypothesis or the sliding model of muscle contraction. The first attempts to correlate data obtained from studies

of the chemistry of contractile proteins while the latter attempts to correlate information attained from physical studies of muscle with a limited number of chemical observations

In the one filament hypothesis contraction is considered to result from physico-chemical changes in a contractile unit consisting of a single filament or molecular strand. The clearest and earliest statement of this hypothesis was from A. H. Meyer²⁷. He proposed a number of models in which attraction and repulsion of charged groups on a helix were responsible for contraction and relaxation. The concept has been developed in a more quantitative manner by Riseman and Kirkwood²⁸ and later by Morales²⁹.

Molecular chains may extend from one Z line to another. Assuming free rotation about chemical bonds and some internal flexibility, the chain would tend to form a coil (see Figure 13). b

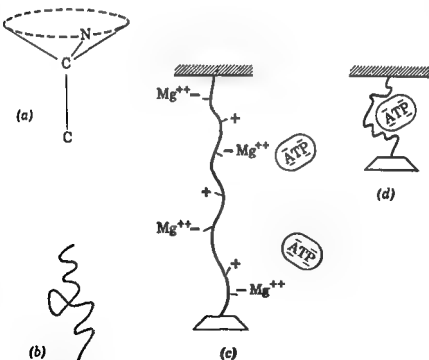


Figure 13 Schema of the one filament or polyelectrolyte model of contraction. Reproduced by permission *Reviews of Modern Physics* 1959 (Morales)

c. d)³⁹ However if charges were distributed along the fiber the tendency to form a coil (entropic forces) would be opposed by an electrostatic force tending to maximize the distances between charges. The resting state or equilibrium is represented by a balance between entropic forces tending to shorten the filament and electrostatic forces tending to keep the molecular chain extended. Magnesium calcium or potassium myosinates might be so extended. Contraction or collapse of such a polyelectrolyte is thought to be the result of a discharge of the fiber by the quadrivalent anions of ATP. The resting state is restored by hydrolysis and disorption of ADP.

Inherent in the polyelectrolyte model is the assumption that the adsorption of ATP and not its hydrolysis is the driving force responsible for changes in the physical properties of the filament.

One of the main objections to such a model is the difficulty in explaining the observation that apparently one molecule of ATP can affect as much as 10 μ g of myosin. This seems to be too large a sphere of action for electrostatic forces. It prompted Szent-Gyorgyi⁴⁰ to invoke long range quantum mechanical forces of energy transmission and excitation. He proposed that in the resting state actomyosin contained stable filled electronic energy levels which were separated from higher unfilled electronic energy levels (semi-conductor). Upon interaction with ATP, certain stable electrons were excited into the high vacant energy levels (conducting level). The spread of this event caused the whole structure to become excited, to depolarize and to go into the contracted state.

Another shortcoming of the polyelectrolyte theory is that it fails to take into account the newer work generalizations of Hill⁴¹ and Fenn⁴². It is for this reason that it should be discarded in its simplest form.

The two filament or sliding model operates on a radically different principle.⁴³ This model attempts to take into account observations on the organization of proteins within the myofibril and the morphological changes which occur during contraction. Stated in its simplest form, upon excitation the thin filaments in the I band slide past the relatively fixed thick filaments in the A band. When the muscle is passively stretched the I band filaments slide

out again. This hypothesis is in keeping with the observation that the width of the A band remains essentially constant at different degrees of stretch and contraction and that the changes in sarcomere length are primarily due to changes in the width of the I band (see Figure 11).

This directed sliding motion during contraction is presumably brought about through the mediation of cross links between the filaments. Such interaction is the structural basis for the reaction between actin and myosin. It is proposed that ATP reacts with the cross linkages and enables the two filaments to crawl or slide past one another.

One of the problems with this model is that during contraction it does not explain why the filaments slide only in one direction and not in the other. The reaction scheme is actually quite symmetrical. Huxley attempts to solve this problem by invoking a spatially asymmetric probability of reaction between the sliding filaments.

Recently Podolsky⁴⁴ has attempted to resolve the differences between the two major theories. He accepts the basic sliding model of the Huxleys but assumes that the thin actin filament is a polyelectrolyte. If discharged the actin filament would coil or fold as the result of entropic forces thereby causing the filaments to slide past each other. He reasons that at rest the actin filament is a positively charged polyelectrolyte and upon excitation some system is activated which deposits ATP molecules on the actin filament. This discharges the filament and it begins to contract, develop tension and draw the two Z lines together. Simultaneously the ATPase on the relatively fixed myosin filament begins to pick off the ATP molecules which tends to restore the charge on the actin filament. Therefore the tension developed and the energy released will reflect an equilibrium between the charging rate and the rate of ATPase activity. This in turn is a function of the relative velocity of motion between the interacting sites on the two filaments.

The models of the Huxleys and Podolsky predict that the work output developed during muscle contraction should increase with a decrease in the relative motion between the two filaments (greater probability of interaction between sites). In this manner

they take into account the well known relationships established by Fenn and Hill for intact muscle. However neither model accounts for the heat of activation of the contractile process.

It is well known that an increase in the initial length of the myocardial fiber is paralleled by an increase in the energy of contraction. Thus it is probable that the initial length determines the velocity of contraction and thereby the extent of interaction between sites on the two filaments.

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Chapter V

CHEMISTRY OF THE NORMAL HEART PART III ELECTROLYTE METABOLISM

Ion Transport : Another aspect of the chemistry of the heart which has received considerable attention in recent years is the transport and accumulation of electrolytes. The heart is similar to other tissues in that the intracellular potassium concentration (K_i) is relatively high and the sodium (Na_i) and chloride (Cl_i) concentrations are low.

There are very good reasons to believe that the quantity of K or Na within the cell is the result of two fundamental unidirectional processes: an inflow (influx) and an outflow (efflux).¹ The quantity of K in the cell may be stated in a rather simple manner by the following relation

$$\frac{dK_i}{dt} = k_i K_o - k_e K_i$$

where dK_i/dt is the quantity of K in the intracellular phase at any time; $k_i K_o$ defines influx and $k_e K_i$ defines efflux. When the concentration of K in the extracellular phase (K_o) and K_i are unity, the transfer coefficients k_i and k_e are the rates of influx and efflux respectively. During the steady state when K_i remains constant, the differential $dK_i/dt = 0$ and efflux equals influx provided all K_i is exchangeable.

The influx and efflux of K may have active or passive components or both. Active indicates that metabolism is involved or that ions are transported against or in excess of an electrochemical gradient. Passive indicates that the driving force is simple diffusion or a favorable electrochemical gradient. In heart recent studies have shown that K efflux is essentially passive while K influx has both passive and active components.² At low K (1.35 mM or less)

K influx appears to be purely passive in response to an active extrusion of Na. At higher concentrations of K active transport components of influx are stimulated. Studies using helium cocaine and changes in temperature show that at 5.4 to 10.8 mM K_0 approximately 43% of K influx is active and 57% is passive.

The chemical mechanisms involved in passive transport or in the preferential accumulation of K and extrusion of Na are not known at the present time. There is good evidence that oxidative metabolism maintains active transport by way of high energy phosphate compounds in particular ATP.⁴

In recent years two specific enzymes have been implicated in cation transport: ATPase and cholinesterase. The latter enzyme hydrolyzes the chemical transmitter acetylcholine. Skou⁵ has studied an ATPase isolated from *Carcinus maenas* nerve. He showed that the substrate most readily attacked by the enzyme was a sodium magnesium ATP complex. An increase in the concentration of Na or a decrease of K led to an increase in ATPase activity. He reasoned that as Na_i increased or K_i decreased active transport mechanisms were stimulated. The relatively large $\Delta F^{\circ}_{hyd. diss.}$ was necessary to drive the process since active transport was essentially an endergonic reaction. More recently Post⁶ has suggested that a membrane ATPase was part of an active transport carrier in the human erythrocyte.

There is now good evidence that cholinesterase plays a role in passive electrolyte transport and thereby is intimately involved in the genesis of the electrical properties of excitable tissue. Acetylcholine the substrate for this enzyme has long been known to affect the passive electrical⁷ and permeability⁸ properties of the heart. It is interesting to note that 50% of this enzyme is located in the L meromyosin fragment of myosin.⁹ Barnett and Palade¹⁰ suggested that this enzyme was localized in the M line. The significance of these observations is not obvious at this time. It should be recalled that ATPase was associated with the H meromyosin fragment.

ATPase and cholinesterase both catalyze hydrolytic reactions and have been localized in cell membranes. Whether these two enzymes are part of a large molecular complex (myosin like) see

above) in the membrane which transports electrolytes is not known with certainty at the present time. If it existed such a complex could be referred to as a cation transport particle (analogous to the electron transport particle isolated from mitochondria by the action of sonic vibrations or detergents (Chapter 3 Mitochondria)).

In Table II we have summarized analyses for several important electrolytes in striated cardiac and smooth muscle.¹² Cardiac tissue contains more water, sodium and chloride and less potassium than skeletal muscle. This suggests a larger extracellular space. The concentrations of magnesium (Mg) and calcium (Ca) in the heart are comparable to those of other tissues. Most evidence indicates that intracellular calcium exists in a bound unionized form.^{1, 15}

The distribution of electrolytes in nodal tissue (atria and ventricles) differ. The analyses of Davies *et al*¹⁶ and Mazel and Holland¹⁷ show that the nodes and AV bundles appear to have a surprisingly large cation excess which is primarily due to the large amount of Na. Mazel and Holland concluded that the intrinsic rhythm of the various regions of the heart was directly proportional to their Na content and inversely related to their K content.

Isotope studies have revealed that myocardial K, Cl and Mg are completely exchangeable within a period of 2 to 4 hours while Na exchanges at a somewhat slower rate.^{1, 18, 8} Ca exchange is of a very slow heterogeneous type and cannot be characterized in a simple manner.^{13, 1} This suggests that Ca exists in several different forms or compartments.

TABLE II
ELECTROLYTE DISTRIBUTION IN SKELETAL, CARDIAC AND SMOOTH MUSCLE

	Skeletal (Dog)	mEq/Liter Cardiac (Dog)	Stomach (Steer)
H ₂ O	14 (°°)	71 (°°)	80 (°°)
Na	25	36	59
K	96	81	91
Cl	111	96	50
Mg	16	16	
Ca	2	2	

The intracellular distribution of these ions within the myocardial fiber is still a matter of conjecture. Mitochondria contain K and smaller amounts of Na, Mg and Ca. Histochemical studies have shown that K is localized in the A bands of cardiac and striated muscle.³ Upon excitation of muscle a redistribution of K occurred and the ion moved from the A bands into the I bands and accumulated at the cell surface.⁴ Micromercurization studies⁵ have localized Ca and/or Mg in membranes A bands and Z lines but not in the I bands.

Electrolytes and Cardiac Contraction. That electrolytes are essential for and modify cardiac contraction has been known since the time of Ringer. Hearts perfused with isotonic NaCl solutions rapidly cease to beat and remain in diastole.⁶ The addition of K delays the Na effect and temporarily restores cardiac contraction. Ca in the absence of K increases the duration of mechanical systole at the expense of diastole and eventually produces a contracture. K in excess prolongs diastole and causes inhibition and arrest.

It is now known that normal cardiac excitation and contraction requires K, Na and Ca in appropriate concentrations. Na is involved in the process of excitability and depolarization. K determines the magnitude of the resting potential and Ca plays a role in excitability and excitation-contraction coupling.

Bioelectric Potentials. In the resting state a potential difference exists between the inside and outside of all excitable cells the interior being negative with respect to the exterior. This potential referred to as the resting or membrane potential is established across a thin barrier (plasma membrane) the thickness of which is of the order of 50 to 200 Å. The heart is no exception and resting potentials of 70 to 80 millivolts have been recorded from single cells in various types of myocardial tissue.^{7,8} A typical transmembrane resting and action potential recorded from a single atrial and ventricular cell are presented in Figure 14. These potentials were recorded by inserting a capillary glass ultra micro electrode (0.5-1.0 μ tip diameter) filled with 3 M KCl into single myocardial cells.

There are distinct features of the transmembrane resting and action potentials that require description. The rising phase or

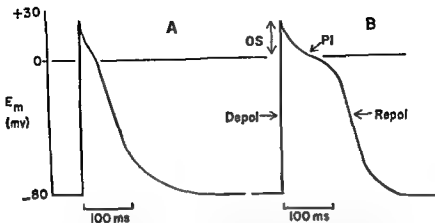


Figure 14 Typical transmembrane potentials recorded from a single atrial cell (A) and ventricular cell (B). E_m membrane potential in millivolts ms millisecond, Depol depolarization Repol repolarization OS overshoot PI plateau

upstroke is referred to as depolarization. That phase where the sign of potential difference is actually reversed is called the overshoot. At the peak spike potential repolarization begins with an initial rapid phase followed by a plateau where the potential is nearly zero and terminates with a faster phase of recovery. The plateau is particularly evident in ventricular cells. Figure 15 shows a transmembrane potential and surface ECG recorded simultaneously from closely adjacent sites. It can be seen that the QRS complex coincides with the upstroke and initial phase of repolarization of the transmembrane action potential. The isoelectric line coincides with the plateau and the T wave with the terminal phase of repolarization.

There is now good evidence that the resting potential of excitable cells results from an asymmetric distribution of K^+ and a membrane selectively permeable to K^+ ions. The membrane is relatively impermeable to other ions. In electrochemical terms the cell may be pictured as a K^+ electrode. The magnitude of the resting membrane potential can be estimated from the Nernst equation

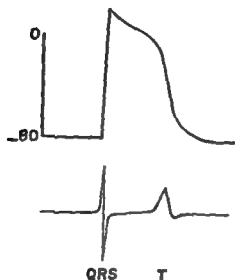


Figure 15 A typical transmembrane potential and ECG recorded simultaneously from adjacent sites

$$E_m = 2.303 \frac{RT}{nF} \log \frac{K_i}{K_o}$$

where E_m is the resting potential or the K equilibrium potential, R the gas constant, F the faraday, n the valence, and T the absolute temperature. When the appropriate units are substituted at 37°C the equation reduces to

$$E_m = 61 \text{ millivolts} \log \frac{K_i}{K_o}$$

The ratio K_i/K_o is of the order of 1.16 to 1.22 in heart muscle which give calculated values of E_m of 73 to 82 millivolts. The measured E_m is usually less than the calculated value.² This indicates that the resting membrane is permeable to ions other than K , such as Na and Cl .²³

Upon excitation the membrane suddenly becomes much more permeable to Na ions than to K or Cl and the cell momentarily becomes a Na electrode. As a result of the opposite orientation of the Na gradient the interior becomes positive (overshoot) with

respect to the exterior. The membrane potential gradually returns to that of the resting state (K electrode) by an initial rapid inactivation of the inrush of Na , an increased outward movement of K ions and possibly by an inward movement of Cl ions. All three of these processes acting singularly or in combination will restore the potential to that of the resting state. Apparently in the heart the plateau results from a delayed inactivation of the inrush of Na or a delayed increase in outward movement of K or a combination of both. It is thought that the duration of the refractory period is determined by the ease of which the inward movement of Na is turned on (availability of the inward Na current) and the rate at which the outward movement of K restores the membrane potential to the resting level. During the interval between each action potential the normal Na_i and K_i are re-established by active transport mechanisms.

Excitation Contraction Coupling The Nature of the Stimulus

The mechanism whereby the action potential propagated along the muscle fiber surface activates the contractile elements is not known with certainty. There is no question that a depolarization or decrease in resting membrane potential is one of the prime factors involved. How this event penetrates into fibers and myofibrils to excite the myofilaments is certainly not clear. It is well known that accompanying activation there are distinct changes in the mechanical properties of the muscle before there is any external sign of tension development. These changes are characterized by an increase in muscle stiffness, resistance to stretch, increase in optical transparency, increase in pH, latency relaxation and heat of activation.²⁴ Whether these changes represent the association of actin and myosin, adsorption of ATP, mobilization of ions (Ca , K , Mg) or hydrolysis of ATP is a matter of speculation. During recent years a number of theories, all involving electrical forces, have been proposed to account for some of the changes observed.

(1) *Electrochemical Gradients and Diffusion* On numerous occasions there have been attempts to explain the events of excitation by assuming the occurrence of diffusion of some essential substance such as Ca , K , or Na from the membrane to the sarcoplasm.

These theories all based on diffusion suffer from a common defect diffusion is not rapid enough ³

(2) *Z Line and Ca Activation Linkage* Recent studies discussed in the preceding chapter indicate that the striations of skeletal and heart muscles are specialized to permit the action of depolarization at the surface to be transmitted quickly inward to the center of the fiber. Tiegs²⁶ in 1934 postulated that the Z line may transmit surface membrane excitation into the interior. By placing fine electrodes along different points of the sarcomere Huxley and Taylor^{3, 35} showed that only depolarization along the Z line was effective in causing shortening of the I band. They pointed out that the extent of spread of the contraction in the I band was directly proportional to the degree of depolarization. This suggested a graded spread of the excitatory process from the Z line and possibly along the thin actin filaments. In addition only certain loci along the continuous Z lines were sensitive to depolarization.

Bianchi and Shanes¹ proposed that certain sensitive patches along the Z line corresponded to sites saturated with calcium which was released into the sarcoplasm during excitation. The quantity and rate of release and its subsequent diffusion longitudinally in the I band may well be related to the magnitude of depolarization. It was pictured as a chain reaction.

Calcium is the only known physiological ion that causes shortening when injected into the muscle fiber at low concentrations³⁶. Since there is evidence that Ca is lacking in the I band it is not unreasonable to assume that the sudden appearance of ionized Ca in this region could either alter the charge or lead to a deposition of ATP on the thin actin myofilaments (Podolsky model). The latter may cause these filaments to fold or coil into the A band. Although there is no experimental evidence for a translocation of Ca in the sarcoplasm during excitation there are considerable data which show that stimulation increases Ca inflow^{3, 37}.

The fact that there is Ca available in the A band suggests that the release of the ion here might permit interaction with the portion of the thin actin filaments which overlap the thicker myosin

filaments and eliminate the necessity of diffusion into the I band. As the I band filaments are drawn into the A band more interaction can occur.

(3) *Endoplasmic Reticulum as an Excitation Conduction Network* Brief mention has been made of the presence in the sarco-plasm of a fine tubular or vesicular network clearly revealed by electron micrographs called the endoplasmic reticulum. As early as 1881 Retzius⁴⁰ suggested an excitatory function for the latter. Porter and Palade⁴¹ proposed that the membrane component of the tubular network might behave as typical cell membranes and relay the action potential from the sarcolemma into the interior of the fiber. In heart Moore and Ruska⁴ have shown that the endoplasmic reticulum appears continuous with the Z lines and extends to intracellular projections of the plasma membrane. The reticulum may not only serve to transmit impulses transversely from the plasma membrane to the Z lines but also longitudinally along the myofibrils to the intercalated discs.^{4, 43}

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Chapter VI

CHEMISTRY OF THE FAILING HEART

Optimum cardiac work can be performed only when the chemical reactions responsible for the release transformation and utilization of metabolic free energy proceed at adequate rates. Interruption of the normal sequence of events at any point may result in cardiac failure. Therefore from the point of view of energetics the problem of where the defect underlying a given type of failure is located in the energy cycle reduces itself to the following question: does the defect concern the release of metabolic free energy or does it concern the utilization of metabolic free energy?

In the preceding chapters we have developed the thesis that in final analysis energy metabolism in the heart revolves around the synthesis and utilization of ATP and like substances. Therefore the problem of the cause of heart failure can be reasked in a somewhat different fashion: is the failure a result of impairment of the generation or of the utilization of free energy of these energy rich phosphate compounds?

Much of the information to be discussed on these and other features of the metabolism of the failing heart can be found in several excellent reviews¹⁻⁶. We shall limit our discussion to those findings which have a firm experimental foundation. Much research in this field is either irrelevant, fraught with technical problems, or carried out under conditions in which its validity at the physiological level is certainly questionable.

Disturbances in Free Energy Release It is only in failures resulting from disturbances in free energy release that a metabolic defect has been reasonably clearly established. Included in this category are failures resulting from anoxia (either due to oxygen

lack or metabolic inhibition) coenzyme lack and hormonal imbalance

(1) *Anoxia* Both acute and chronic anoxia lead to heart failure. These failures are usually characterized by relatively high cardiac outputs with low mechanical efficiencies. Many of the oxidative enzymes associated with the Krebs citric acid cycle and the electron transport system deteriorate rather rapidly following periods of relative anoxia.⁷ These changes are usually associated with fragmentation, swelling and distortion of the normal ultra structure of the myocardial sarcosomes.^{8,9}

A number of studies have shown that the decline in work capacity associated with anoxia is roughly proportional to the decline of phosphocreatine content and later to a fall in ATP concentration.^{10,11} Upon readmission of oxygen both the work capacity and content of energy rich phosphate compounds return to normal. Furthermore failures resulting from metabolic inhibition (cyanide, 2,4 dinitrophenol) are also characterized by a fall of phosphocreatine and later by a decline of ATP.¹²

(2) *Coenzyme Lack and Hormonal Imbalance* Probably the most important example of myocardial failure resulting from coenzyme lack is that seen in the disease beriberi resulting from inadequate amounts of thiamine in the diet.¹³ The heart and other tissues undergo a depletion of co-carboxylase, one of the coenzymes concerned with the conversion of pyruvate to acetyl coenzyme A.¹⁴ In this condition the oxidation of carbohydrates cannot proceed to completion.

As would be expected in thiamine deficiency the myocardial extraction of pyruvate and lactate is depressed.¹⁵ Lactate extraction is apparently inhibited more than pyruvate probably as a result of the adverse effects of elevated pyruvate levels on lactic acid dehydrogenase. Glucose extraction is also reported to be abnormally low.

Another type of failure of considerable academic and clinical interest is that resulting from thyrotoxicosis. The mechanism of the metabolic derangement in thyrotoxic heart disease is not known. The available evidence points to a defect in free energy release. Oxygen consumption of the heart is usually elevated but

energy utilization is not improved and the overall efficiency is low.¹⁷ The threshold for glucose extraction by the intact thyrotoxic heart is elevated and the extraction coefficients for pyruvate and lactate are reduced. Observations on fatty acid metabolism suggest that the heart in thyrotoxicosis depends to a great extent on the utilization of these substances for energy production.

Myocardial ATP and phosphocreatine are also exhausted in thyrotoxicosis.¹⁸⁻²⁰ The decrease in ATP is probably related to thyroxine's effect on oxidative phosphorylation.²¹

As with cardiac failure induced by anoxia that resulting from thiamine deficiency and thyrotoxicosis are relatively resistant to the action of the cardiac glycosides.

Disturbances in Free Energy Utilization There is considerable evidence that congestive heart failure may occur even in the presence of normal free energy release. Classical congestive heart failure may be thought of as resulting from a defect in free energy utilization. It is characterized by a low cardiac output despite a normal or increased oxygen consumption and substrate utilization and normal contents of phosphocreatine and ATP.³ Such a situation is found in failures resulting from chronic valvular and hypertensive heart disease in man, spontaneous failures in the heart lung preparation and failures induced by ion excess and certain narcotics (nembutal, ethyl urethane, etc.).³ They all respond to the digitalis glycosides.

The basic defect in congestive heart failure appears to be an inability of the myofibril to assimilate phosphate bond energy. Whether this is a result of abnormal physico-chemical properties of the contractile protein actomyosin or to the loss of essential electrolytes or metabolites has not been clearly established.

The failure to demonstrate a defect in free energy release in these failures served as stimulus for the search for causes at the molecular level of the contractile apparatus. During the past decade a number of reports have appeared dealing with the extractability and physico-chemical properties of actomyosin obtained from hearts in congestive failure.

Kovacs²² was the first to develop methods for the extraction of myosin and actomyosin from the heart. The techniques have

been refined by Benson *et al*^{3,4} and later by Dettli and Bing. Benson *et al* report that in dogs with chronic heart failure secondary to surgically produced valvular disease the concentration of actomyosin is decreased. These changes were found in both ventricles despite the fact that the increased work load was usually limited to the left ventricle. A decreased viscosity per unit of actomyosin as well as a reduced response of the viscosity of actomyosin solutions to ATP was also reported. They felt that these changes resulted from actomyosin existing in a partially dissociated state which could be the result of excessive stretching of the myocardial fibers. Somewhat similar findings have been reported by Dettli and Bing⁵ and Olson *et al*.⁶

More recently Ellenbogen *et al*⁷ report further studies on the physicochemical characteristics of myosin. Cardiac myosin isolated from dogs in congestive failure was found to have a molecular weight three times (740 000) that of normal hearts (229 000) and the longitudinal dimension of the particles was approximately twice that of normal myosin (1200 Å compared to 680 Å). Apparently during the development of failure myosin molecules aggregate to form large molecular complexes. These authors also interpret their findings as resulting from chronic stretch of the myocardium which leads to irreversible changes in the size and shape of cardiac myosin. More will be said of these changes later.

Electrolyte Metabolism The possible relationship of electrolyte disturbances to the mechanism of heart failure has engendered extensive discussion and prolific writing in recent years. For some time it has been known that changes in the electrolyte composition of cells may occur in congestive heart failure.^{8,9} They usually consist of an increase in cell sodium and a decrease in cell potassium. The question immediately arises as to whether the observed changes in the heart are of any basic importance in the genesis of the failure or do they merely reflect the response of the heart to an abnormal volume and distribution of fluids and electrolytes.¹⁰

The situation is further complicated in most clinical conditions since digitalis usually has previously been administered to patients with myocardial failure. As we shall show later the glycosides cause a release of K and gain of Na in the heart. However

the elaborate and careful studies of Clarke and Moscher²¹ clearly show that an abnormal distribution of electrolytes occurs in hearts of patients who have received no glycoside therapy.

In light of the known role of electrolytes in myocardial contraction it is conceivable that a defect in electrolyte metabolism may be a causative factor in congestive heart failure. However the available evidence to date suggests that the abnormal Na and K metabolism in this condition is secondary and does not play a primary role in the genesis of failure.

A demonstrable defect in calcium metabolism would be far more significant as this ion is absolutely essential for the process of contraction. It is indeed significant that Kapeller and Kutschera Aichenbergen² demonstrated a diminished calcium content in hearts in congestive failure resulting from chronic valvular disease. Part of the Ca was bound to the lipid fraction. The decreased Ca content of the insufficient heart was frequently most pronounced on the side bearing the greatest fractional load. With the development of more refined techniques for estimating bivalent cations these findings merit further study.

Hypertrophy Morphological Considerations Probably the most important compensatory mechanism involved in heart failure is that of hypertrophy. The failed heart may increase its mass by as much as six times and its effective work by as much as twenty times.²² Harrison²⁴ has postulated that a decisive factor in the development of congestive heart failure is anoxia of the myocardium resulting from compensatory hypertrophy. He reports that the thickness of the hypertrophied fibers does not permit adequate oxygen diffusion. Weirn¹ reports that the total capillary cross sectional area per unit volume of hypertrophic heart muscle is 1/3 as large as that in normal heart muscle. On the other hand Dock²⁵ points out that the coronary flow in cardiac hypertrophy without coronary disease is more than adequate to provide oxygen in excess of the requirements of even the thickest fibers. Bing *et al*²⁷ report that in patients with congestive heart failure resulting from valvular disease oxygen consumption is either normal or slightly elevated.

The most distinctive morphological feature of hypertrophy is the increase in the diameter of the individual myocardial fiber with

little or no increase in capillary supply.³³ Linzbach³⁴ has shown that in mild hypertrophy there is probably no increase in the number of fibers. This physiologic hypertrophy is usually seen in hearts weighing up to 500 grams. However both Linzbach and Lowe and Bate³⁵ pointed out that when this weight is exceeded the thickened fibers split longitudinally producing a net increase in the number of fibers. Apparently with further hypertrophy the daughter fibers also increase in size and split after reaching a critical width.

Recent electron microscopic studies have revealed some interesting ultrastructural correlates to the changes described above. Molbert and Iijima⁸ observed that with increasing hypertrophy the myofilaments enlarged and became thicker. At a critical size they split into daughter filaments. These investigators also described changes in the sarcosomes such as swelling, diminution of the cristae and shrinking of the inner of the outer two sarcosomal membranes. Further details on the endoplasmic reticulum, Z lines and intercalated discs have been described by Bryant *et al*.⁹

Hypertrophy: Chemical Considerations It should be pointed out that many of the studies reviewed in the present chapter on energy metabolism in the failing heart were carried out on hypertrophic hearts. There have been only a limited number of studies emphasizing the changes in the chemical composition accompanying hypertrophy.

Schulze and Sudhof¹⁰ report an increase in the phospholipid fraction and a decrease in glycogen. Sobel and Cohen¹¹ analyzed for a number of protein fractions in the hypertrophic rat heart. Increases occurred in all fractions analyzed but in a disproportionate manner. The largest increases were observed in the myoglobin, actomyosin and mitochondrial fractions. The authors suggested that these changes represented an adaptation to the increased demands placed on the heart.

The increase actomyosin content may explain the observed increase in ATPase activity reported by Rossi and Durrant-Morris.⁴ The latter appeared to be related to sarcosomal damage; however oxidative phosphorylation was not changed during the period of observation. The same authors¹² also report that an initial increase

in ribonucleic acid occurred in the tissue homogenates and isolated mitochondria this returned to normal in four months. The desoxyribonucleic acid per unit weight was not modified after observation for eight months but the total amount increased due to the increased number of nuclei. Nowy *et al*⁴⁴ report only a slight increase in ribonucleic acid, a slight decrease in desoxyribonucleic acid and no changes in total phosphate or inorganic phosphate in rabbit hearts after six months of hypertrophy.

These findings suggest that an increase in protein synthesis occurs during the initial stage of hypertrophy as one would expect. It is of interest to note that cardiac hypertrophy does not occur in hypophysectomized rats.⁴ Treatment of such rats with growth hormone restores the ability of the heart to undergo hypertrophy.

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Chapter VII

ACTIONS OF DIGITALIS

Withering³ was the first to recognize that digitalis had profound effects on the motions of the heart. He actually developed successful methods for the use of this agent in treating dropsy, a disease syndrome which we recognize today as congestive heart failure. In 1882 Schmiedeberg determined the basic actions of the digitalis substances and began a study of the action of purified glycosides. Fraenkel introduced strophanthin in glycoside therapy of heart disease in 1905 after preliminary animal experimentation.⁴

The rapid progress made in muscle chemistry and energetics of muscle contraction during the period 1910 to 1950 served as a stimulus for a search for the mode of action of these agents at the chemical level. During this interval a bewildering number of studies have appeared. Interest is continuing at an accelerated rate.

From a chemical and pharmacological point of view the most important site of action of the cardiac glycosides is unquestionably the myocardium. The systolic force of muscle contraction of the failed or hypodynamic heart is increased. In fact all the effects on the heart can be explained by an action on this parameter. Admittedly these agents have effects on cardiac reflexes⁵ and renal excretion of electrolytes. However the magnitude of the changes induced are not sufficient to account for the therapeutic effectiveness of the drug.

In the preceding chapter we noted that failures resulting from a defect in free energy release do not respond effectively and efficiently to digitalis. Beneficial effects of the drug were only obtained in failures resulting from disturbances in free energy utilization. These findings indicate that digitalis is active by virtue of its ability to improve the utilization of metabolic free energy.

In the present chapter we shall briefly review some of the pertinent evidence which substantiates the above hypothesis

Overall Metabolism One of the most distinctive and easily reproducible effects of digitalis on overall metabolism is its action on oxygen consumption of myocardial tissue. In 1912 Kohde and Ogawa¹ reported that strophanthin depressed O_2 consumption of the frog heart. In 1930 Eismayer and Quincke obtained considerable increases in O_2 uptake with a very low concentration of strophanthin which had no influence on the work output of the heart. In the same year David² noted that strophanthin caused a similar increase in respiration of frog atria which was later followed by a decline to below normal levels. Since the frequency of beat was lowered and the amplitude of beat not greatly augmented this stimulation of oxygen consumption seemed to be a primary effect.

This effect on oxygen consumption has been observed on numerous occasions in steadily respiring cardiac tissue slices.³⁻¹¹ The effect is observed at low pharmacologically active concentrations. Increases of oxygen consumption are usually observed initially (30 minutes to 1 hour) followed later by a profound depression. This effect is blocked by increasing the K^+ concentration¹² but occurs in the absence of Ca^{++} . Significant changes in the respiratory quotient have not been noted.¹⁰ In higher toxic concentrations the glycosides increase the O_2 consumption of brain slices.¹⁶

The intact cardiac muscle fiber is required for the above effect. In concentrations 100 times those exerting an effect on cardiac tissue slices ouabain has no effect on fortified or unfortified homogenate respiration.¹⁰ Furthermore the drug has no effect on the respiration of normal mitochondria or those whose respiration has been depressed with metabolic inhibitors.^{4, 16}

This effect on respiration has not been universally accepted. For example Bing and associates have found that strophanthin did not modify coronary blood flow or aerobic metabolism in either normal subjects or patients with cardiac insufficiency.¹

At the present time there is no satisfactory explanation for this increase and subsequent decrease in the rate of respiration. Increased cell permeability^{14, 19} either to exogenous essential sub-

states or to normal intracellular metabolites has been invoked on several occasions

Finklestein and Bodansky¹¹ pointed out that the observed increase in oxygen consumption may well result from the development of a contracture that occurs in the presence of the glycosides. In fact Victor⁹ reported that ouabain in rather high concentrations greatly increased O_2 consumption and he found ventricular muscle firmly contracted at the end of the experiment. This hypothesis could not explain the increased oxygen consumption of brain slices induced by digitalis.

Recently it has been shown that ouabain increases Ca^{2+} influx.¹² It is well known that Ca^{2+} is an activator of ATPase, particularly myosin ATPase. The increased ATPase activity that might occur under these conditions would lead to an accumulation of free PO_4 and ADP. Both of these agents are activators of the glycolysis and oxidative phosphorylation reactions. The elevated oxygen consumption may well be the result of such a sequence of reactions. In keeping with this hypothesis is the finding of a fall of creatine phosphate and later ATP in hearts after prolonged treatment with ouabain.¹³ On the other hand, this idea would not explain the subsequent decrease in respiration.

Free Energy Release. An almost overwhelming and bewildering number of studies on the effects of digitalis on the metabolic patterns involved in the release of free energy have appeared during the past 30 years. Much of the data is conflicting and difficult to analyze. Therefore we shall limit our discussion to studies that illustrate the problems involved.

Gremels¹⁴ reports that digitalis increases glucose utilization at constant work levels in the heart lung preparation. Apparently a sharp rise in lactic acid uptake occurred after administration of nontoxic doses of ouabain to dog heart lung preparations. Following the administration of digitalis myocardial glycogen content has been reported to be increased,¹⁵ decreased,^{17,18} or not significantly altered.^{19,20} Bing and associates report that digitalis has no effect on myocardial extraction of glucose, lactate, pyruvate or ketone bodies in the normal and failed heart.²¹

Averbeck²² states that cardiac glycosides accelerate the anaero-

bic oxidation of glucose hexose diphosphate glycerophosphate and lactate Succinate oxidation was reported to be increased in minced cardiac muscle

The cytochrome oxidase system appears to be unaffected by digitalis^{10,11} Neither does the presence of cardiac glycosides have any influence on the electron transport system¹⁰ nor on the efficiency of oxidative phosphorylation¹²

Therapeutic concentrations of these agents have no effect on the ATP and phosphocreatine content of the normal or failing heart⁹ Higher toxic doses cause a rapid depletion of the energy rich phosphate stores^{3,31} This fall in energy rich stores has been interpreted by some as resulting from an inhibition of ATP synthesis relative to ATP breakdown However it was pointed out above that these agents have no demonstrable effects on oxidative phosphorylation

It is highly probable that the loss of energy rich stores may be the consequence of an increase in the rate of breakdown of ATP It is conceivable that the activity of the Ca activated myosin ATPase of heart might be increased by the glycosides perhaps through mobilization of calcium as pointed out earlier

Free Energy Utilization There is considerable evidence available which suggests that digitalis has effects on the physico-chemical properties of the ATP actomyosin system In 1949 Mallov and Robb³² showed that the shortening of actomyosin threads induced by ATP was increased by digitalis This finding has been confirmed by Bowen³⁶ Horvath *et al*³⁷ reported that the rate of polymerization of C actin was enhanced by small amounts of digitoxin Similar observations were made by Wollenberger³⁸ who compared the effects of a number of active and inactive glycosides However he concluded that there was no correlation between the *in vivo* action and the effect on isolated protein Waser^{39,40} found that pharmacologically active glycosides decreased the thixotropy of actomyosin and increased the K binding capacity of this protein Inactive glycosides had no effect

Waser and Volkart⁴¹ reported that several glycosides reduced the viscosity of actomyosin solutions but not of myosin and did not affect the degradation of ATP by myosin solutions Dettli and

Bing⁴⁴ found that actomyosin bands prepared from hearts in congestive failure showed reduced contractility when compared to those prepared from normal hearts. Both preparations were unaffected by digoxin or by Ca alone. However, in the presence of digoxin plus Ca (10^{-4}M) the contractility of the protein isolated from hearts in congestive failure returned to normal. The glycoside had no effect on the glycerol extracted heart fibers. It should be pointed out that the latter preparations have no functional membranes and are electrically inexcitable.⁴

Electrolyte Metabolism It has long been known that the systolic action of digitalis is influenced by the ionic environment. The NaCl content of the suspending medium does not seem to affect the action directly for it may be reduced considerably without changing the action of digitalis providing the osmotic pressure is maintained with sucrose. It is of interest to note that Daly and Clark⁴⁵ observed that the effects of decreased Na showed remarkable similarity to those of strophanthin. An increase of K lessens while a decrease enhances the systolic action of digitalis. Apparently an increase in pH favors the actions of strophanthin.

Special interest is attached to the relation of calcium to digitalis action. It has long been known that Ca increases the force of contraction and promotes the development of contracture in a manner similar to digitalis. Furthermore weakened contractions of hearts beating in Ca deficient media can be restored to normal by the addition of strophanthin. When the calcium is reduced nearly to zero the systolic action is greatly impaired.

In 1919 Korschegg⁴ working in Loew's laboratory suggested that strophanthin could substitute for Ca . Loew⁴ developed the thesis further and pointed out that the glycosides may activate the Ca of the heart or sensitize the muscle to the Ca it contains.

These observations suggest that digitalis might have actions on electrolyte metabolism. However it was not until the studies of Calhoun *et al*⁴⁶ that concerted efforts were made to determine whether digitalis had demonstrable effects on electrolyte metabolism in the heart. The finding that hearts of patients dying of congestive failure were abnormally low in K content led them to suspect that digitalis might have effects on K metabolism in the

heart. In a well controlled series of experiments on dogs the observed that toxic and fatal doses of digitalis markedly diminished the K concentration in the heart but not in skeletal muscle.³⁰

Since these original observations a large number of papers have appeared which show that digitalis in therapeutic or toxic doses decreases myocardial K.³¹⁻³³ The loss of K is accompanied by a corresponding increase of cell Na.

Wood and Moe³⁴ showed a positive correlation between the increased mechanical efficiency and rate of loss of myocardial K. This thesis has been expanded into a rather elaborate theory by Hajdu³⁵ to explain the mode of action of digitalis.

Recent studies on erythrocytes³⁶ and cardiac muscle³⁷ indicate that the loss of K and gain of Na result from an inhibition of active Na efflux and K influx. Solomon *et al.*³⁸ suggest that digitalis competes in a reversible manner with Na and K for the active transport carriers. Glynn³⁹ reports that digitalis also blocks passive transport of Na and K while Holland and Klein⁴⁰ present data suggesting that digitalis under certain conditions increases passive membrane permeability to these cations. Recently Holland and Sekul⁴¹ have shown that digitalis increases Cl influx.

The chemical mechanism involved in the action of cardiac glycosides on active transport is at the moment incompletely understood. As pointed out in an earlier chapter there is evidence that a membrane ATPase is part of the active transport carrier system. Recently Post⁴² observed that in hemoglobin free erythrocyte membranes ATPase activity was approximately doubled by the addition of both Na and K together but not by either ion separately. The increase in activity was prevented by ouabain (10^{-5} M).

Earlier Holland, Greig and Dunn⁴³ observed a similar competition between K and ouabain in the acetylcholine cholinesterase system. The concentration of ouabain used was higher by a factor of ten than that employed in the above studies. In an earlier chapter it was postulated that the two enzymes might be part of a large molecular complex in the cell membrane controlling active and passive movement of Na and K. Therefore it is possible that the glycosides produce their effects on active and passive movement of cations by interaction with this molecular system.

Interest in Ca and its possible relation to the mode of action of digitalis has been recently revived. Wilbrandt and Cuvierel⁶³ have described experiments which appear to show that digitalis prevents the loss of myocardial Ca probably by depressing Ca outflow. Later Thomas *et al.*¹ and Hollind and Sekul⁶⁴ showed that ouabain had no demonstrable effect on Ca efflux but markedly increased Ca influx. The latter authors showed that this effect was enhanced by an increase of Ca and blocked by an increase of K in Ringer's solution.

Niedergerke⁶⁵ presents evidence that much of the calcium involved in contraction is very rapidly exchangeable and probably involves that in the membrane. The fast exchanging fraction represents only a small portion of the total (1 to 2%)⁶⁶. The more slowly exchanging calcium (bound form) is not involved in rapid changes in contractility. It is possible that digitalis affects only the rapidly exchanging component. This might explain why some investigators report systolic effects of cardiac glycosides in media which were thought to be free of calcium. Hajdu and Leonard⁶⁶ suggest that the increased Ca influx may result from the great loss of K that occurs in the presence of digitalis.

The cardiac glycosides also have effects on the electrical properties of the heart—an action probably resulting from an effect on membrane permeability. Initially these agents lengthen the duration of the transmembrane action potential by increasing the plateau. Later a shortening and spiking of the potential is observed. This is followed by a loss of overshoot, an increase in membrane resistance, and after long exposure a decrease of membrane resistance.^{67, 68}

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Chapter VIII

THE NATURE OF CONGESTIVE HEART FAILURE AND THE MODE OF ACTION OF DIGITALIS

The analysis in the preceding chapters revealed that only in those cardiac failures resulting from metabolic inhibition vitamin deficiency or hormonal imbalance could a metabolic defect be implicated as the cause of reduced mechanical efficiency. A disturbance in free energy release seems reasonably clear. Such failures do not respond well to digitalis and only by alleviating the precipitating factor can they be effectively corrected.

We presented strong evidence that in those failures which respond to digitalis the cause of mechanical inefficiency seemed not to reside in a reduced energy supply but in an inability of the myocardial fiber to transform metabolic free energy into mechanical work. The most important examples in humans are congestive failure resulting from chronic hypertensive and valvular disease and certain experimentally induced failures in the dog heart lung preparation. Only these types will be considered in the present chapter.

Theories of Congestive Failure There is now general agreement that the development of congestive failure results from a diminishing work capacity per unit weight of myocardial tissue.¹ Apparently when this parameter falls below a critical value symptoms of frank congestive failure appear.

Probably the oldest and most widely accepted theory to account for the events accompanying the development of congestive heart failure is that due to Frank and Starling.² These investigators showed that the contractile energy of the heart was a function of the initial length of the myocardial fiber. Contractile force increased in direct proportion to initial fiber length up to a certain critical

one further lengthening resulted in diminished contractile energy. Starling further proposed that congestive failure resulted when the fibers were stretched beyond this critical length due to excessive loads being placed upon the heart. Therefore according to the views of Frank and Starling excessive stretch of the myocardium is of prime importance to an understanding of the mechanism of congestive failure.

The validity of this concept has been recently challenged particularly by Katz.⁴ He believes that the initial length of the fiber is not the sole factor governing the release of contractile energy; the velocity of contraction and neurohumoral events should also be considered. However the carefully controlled studies of Burnoff⁵ clearly show that under any given set of hemodynamic conditions a direct proportionality exists between contractile energy and diastolic volume. Thus the validity of the Frank-Starling principle seems to be well established at the present time.

The importance of the velocity of contraction as a factor controlling the release of energy should not be minimized. In skeletal muscle it has been shown that the slower the velocity of contraction the greater the rate of energy release.⁶ Furthermore skeletal muscle liberates extra energy for work⁷ (Fenn effect). Such crucial findings have not been tested on the heart and their role in controlling the work of the heart is not clearly evaluated.

Harrison⁸ postulated that a decisive factor in the development of congestive failure was relative anoxia resulting from compensatory hypertrophy. He thought that the thickened myocardial fiber limited the diffusion of oxygen and essential substrates. However in Chapter VI we presented arguments that invalidate this thesis.

There is considerable evidence available which suggests that congestive heart failure is the result of changes in the physicochemical properties of the contractile proteins. This is certainly in keeping with findings of a diminished actomyosin concentration in failed hearts as well as the isolation of cardiac myosins having abnormal physical properties. In addition the contractility is reduced in glycerol extracted fibers prepared from hearts in congestive failure. One of the major obstacles to the acceptance of such a theory is that muscle protein changes were found in both ventricles

cles despite the fact that the increased work load was either limited to the right or left ventricle.⁸

Another explanation for the reduced mechanical efficiency is that there is some alteration in the intracellular ionic environment which results in a diminished contractility. It was pointed out earlier the hearts of patients dying of congestive failure are low in K and high in Na. This finding was complicated by the fact that many of the patients had received cardiac glycosides, agents known to produce a comparable electrolyte distribution. However in the studies of Clarke and Mosher¹⁰ a similar picture was observed in patients not receiving digitalis. In fact these authors believe that digitalis elevates the low K levels back to normal.

It is still a controversial matter as to whether the reduced K and elevated Na is the cause or result of cardiac insufficiency. Similar changes have been reported in skeletal muscle of patients with non-cardiac edema.¹¹ McDowall *et al*¹ believe that cardiac failure results from the inability of the heart to extrude Na actively. The accumulation of Na has deleterious effects on the contractile apparatus. This hypothesis would explain why decreasing the Na content of the medium increases the contractile strength of heart muscle. However Niedergerke and Harris¹² have recently shown that a decrease of Na increases the uptake of Ca.

The role of Ca ions in congestive failure should be considered because of their well known effects on muscle contractility. Unfortunately little or no attention has been given to this problem in recent years. This is probably a result of the fact that total tissue calcium is difficult to determine and that calcium exists in several forms in tissue. In this connection it should be noted that Kapeller and Kutschera Aichenbergen¹⁴ found a diminished Ca content in hearts in congestive failure resulting from chronic valvular disease. The decreased Ca was most pronounced on the side bearing the greatest work load.

Of the various theories proposed over the past 50 years to explain congestive failure that of Frank and Starling is the most reasonable and is in keeping with recent studies on muscle contraction.

Earlier we discussed evidence which indicated that heart and

skeletal muscle contract to perform work by an interaction between sites on overlapping myofilaments in the sarcomere. Apparently the interaction is between ATPase sites on the dense myosin filament in the A band and ATP bound to actin in the thin I band filament. The reaction permits the actin filament to slide past the more rigidly fixed myosin one. The energy released in the form of work is determined by the extent of interaction which in turn is controlled by the relative velocity of motion between filaments and the concentration of ionized calcium: the slower the relative motion the greater the interaction. Ionized Ca^{++} is thought to act either by strengthening or establishing cross links between the filaments or by increasing ATPase activity and thereby enhancing the probability of interaction.

If the sliding model is a correct representation of the events associated with normal contraction then contraction should be weakened or cease when the sarcomere is stretched beyond a critical length (about 3.5μ). This length should be the sum of the lengths of the A (1.5μ) and I (2.0μ) band filaments. Huxley and Percey¹ showed that a muscle fiber with a sarcomere length of 3.5μ shortened greatly on stimulation but if stretched to 3.6μ it did not shorten at all. This is indeed a relatively narrow range. Furthermore they pointed out that when an intact muscle fiber is stretched the sarcomeres in the middle of the muscle bundle extend more than those at the ends.

It should be recalled that Starling felt that congestive failure resulted from the myocardial fiber being stretched beyond a critical length. Therefore in light of the sliding model it is conceivable that excessive stretch may so displace the myofilaments relative to each other that interaction is decreased or absent resulting in diminished contractile strength. When a sufficient number of the myofibrils are involved frank congestive failure results.

Excessive stretch may lead to other changes that contribute to the severity of failure. For example excessive stretch may distort the physicochemical structure of the actomyosin molecule so that its ion binding capacity is disturbed and essential electrolytes are lost. It is well known that ionized calcium is a potent activator of myosin ATPase activity. According to the sliding model the

probability of interaction of the ATPase site on the thick myosin filament with bound ATP on the actin filament would rise or fall with an increase or decrease of ATPase activity. Thus a decrease of ionized Ca in the environs of the contractile elements as is reported to occur in congestive failure would reduce the rate of energy released with each contraction.

The Mode of Action of Digitalis There is no doubt that digitalis improves the mechanical efficiency of the failing heart. At the present time there is good evidence that this results not from an action on free energy release but by correcting a defect of free energy utilization.

We presented evidence that cardiac glycosides act on the contractile protein actomyosin as well as on the cell membrane. We are of the opinion that the two basic actions are inseparable and that the glycosides act through a common mechanism the transport and distribution of electrolytes across the cell membrane.

It is very difficult to implicate changes in Na and K transport in the mode of action of digitalis as these agents produce changes in ion distribution similar to those that occur in congestive failure. The effects of digitalis on Ca exchange appear far more significant. Present evidence indicates that digitalis increases the available ionized calcium in the vicinity of the contractile apparatus either by increasing its rate of inward movement across the membrane or by releasing it from binding sites in the membrane or myoplasm.

By increasing the available ionized Ca in the immediate vicinity of the contractile proteins digitalis would act to enhance the existing interaction between these proteins. Ca may function by establishing more cross links between the overlapping filaments or it may act to stimulate ATPase activity which would increase the probability of interaction. The sum total effect is to make the interaction between the contractile proteins the same per unit time in the failing heart as that in the normal heart even though the overlap of these filaments is considerably less. Figure 16 illustrates this important point.

It is well known that the systolic effects of the cardiac glycosides are only temporary. Apparently this is not the result of the development of tolerance as the toxic manifestations are evident

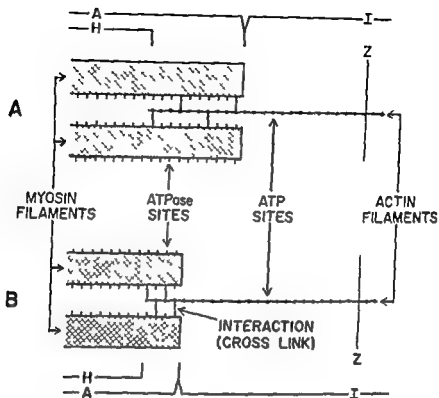


Figure 16 Hypothetical interaction between filaments in normal (A) and failing (B) myocardium

at the dose levels originally employed. According to the sliding model, digitalis glycosides would be effective only as long as sufficient overlap existed between the actin and myosin filaments. With further stretch, interaction diminishes or may even be eliminated in some fibers. When interaction in a sufficient number of fibers is absent, intractable congestive heart failure would become evident and the beneficial effects of digitalis would disappear.

We feel that this is a rather provocative hypothesis as to the nature of congestive failure and the mode of action of digitalis. At the present time there are no studies available on the ultrastructural changes in the myofibril accompanying the development of heart failure. Additional research, preferably along the line of the

Huxleys will be needed before we have a clear and exact picture of the nature of the physico-chemical events responsible for this intriguing clinical syndrome

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Chapter IX

FIBRILLATION

A monograph on the chemistry of heart failure would hardly be complete without a consideration of fibrillation. It is well known that fibrillation is usually the terminal event in many types of failure both acute and chronic.

By 1900 ventricular fibrillation had been described by Erichsen (1812),¹ Hoffa and Ludwig (1850) and Langendorff (1894)² in his perfused heart preparation. Physiologic interest was limited and its clinical counterpart was not suspected. Heimg³ in 1906 at the Physiological Congress in Heidelberg demonstrated that an excess of potassium inhibited fibrillation of the perfused heart and that a subsequent perfusion with normal Ringer's solution restored the rhythm to normal.

There are now available a number of chemical and physical methods for producing fibrillation. In atria Rothberger and Winterberg⁴ in 1910 produced it by rapid stimulation. Lewis and collaborators⁵ in 1920 induced it by rhythmic stimulation rising to a critical rate between 350 and 500 per minute and Andrus and Carter produced it in dogs by a single stimulus at the end of the refractory period during vagal stimulation.

In 1917 Meek, Hathaway and Orth⁶ produced ventricular fibrillation by the administration of epinephrine in conjunction with cyclopropyl anesthesia. Nahum and Hoff⁷ in 1910 produced atrial fibrillation with the application of acetyl β methylcholine to localized regions on the atrial surface. Sherf, Romano and Ferrarini¹⁰ produced both flutter and fibrillation by injection of a dilute solution of aconitine or by applying crystals of aconitine to the atrial surface. West, Turner and Loomis¹¹ noted occurrences of arrhythmias when the interval between stimuli was such that the

latter fell in the relative refractory period. The arrhythmias lasted a few seconds to several minutes and were reported to occur regularly in the presence of acetylcholine.

Burn, Vaughan Williams, and Walker⁷ described a method by which fibrillation could be induced and turned off at will by stimulating atria of the dog heart lung preparation perfused with acetylcholine. Later Holland and Burn¹¹ produced the arrhythmia in isolated rabbit atria by stimulating at high frequency in the presence of acetylcholine. The production of fibrillation in this preparation was dependent upon three factors: (1) the presence of a low concentration of potassium in the medium; (2) the presence of a high concentration of acetylcholine; and (3) brief electrical stimulation at a high rate (600 to 1200 per minute).

Theories of Fibrillation The first and oldest explanation of fibrillation was offered by Engelmann¹² in 1893. He proposed the existence of a number of simultaneous stimuli producing force in the heart, each having its own intrinsic rhythmicity and operating independently. Engelmann's theory was based on the assumption that fibrillation was the result of the heart existing in a state of increased excitability. Winterberg¹ extended this concept and proposed that because of the increased excitability the heart fibers became independently rhythmic. Engelmann's hypothesis was soon abandoned when it was shown by McWilliams¹³ that decreased excitability was also an important factor in the causation and maintenance of the disorder.

Kothberger and Winterberg¹ in 1914 proposed another theory according to which fibrillation was due to an extremely rapid unifocal tachysystole. Fibrillation in their view was merely a tachycardia of exceptionally rapid rate. They envisaged the existence of a single ectopic focus of high inherent rhythm which discharged repetitively and therefore acted as a pacemaker. This theory became known as the ectopic focus theory of fibrillation.

At about the same time Mines¹ and Carrey¹⁴ proposed another mechanism, the so-called circus movement theory of fibrillation. This theory is based upon the re entry of an impulse into an area of tissue that had responded previously to the same

excitation wave. The existence of a circus movement of excitation was experimentally demonstrated by Garrey.

This hypothesis had its origin with the evidence presented by Porter⁹ that an intramuscular block would deflect an impulse and cause it to pursue an abnormal path. Mayer¹ in 1908 first observed that if an excitatory wave started in one direction only in a ring of muscular tissue of the jelly fish, it would travel for a prolonged period of time. Garrey¹⁰ and Mines¹¹ demonstrated this phenomenon in rings of atrial and ventricular muscle. Lewis¹² advanced the idea that circus movement would arise and pass around a ring of atrial tissue surrounding the junctions of the inferior and superior vena cavae. However the existence of such a muscle entity has been disputed.

Recently the ectopic focus theory of fibrillation has been revived primarily as a result of the studies of Scherf¹³ and Prinzmetal¹⁴ and collaborators. Scherf was able to produce atrial fibrillation by topical application of aconitine to the exposed atrial surface of the dog. The ectopic focus theory drew strong support when it became evident that aconitine induced fibrillation could be arrested by cooling the site of application.

Fibrillation was also produced by the action of acetylcholine. It differed from that produced by aconitine in that a similar inhibition at the site at which acetylcholine was applied did not arrest the condition. However simultaneous inhibition of the SA and AV nodes usually stopped fibrillation produced by both topically applied acetylcholine and by means of electrical stimulation.

This difference in response suggests that the mechanism of fibrillation is not due to a single agency. At least in the case of atrial fibrillation two forms can be distinguished, one with a single focus and the other with multiple sustaining foci. The two forms however cannot be differentiated upon inspection of the electrocardiogram. Katz and Pick¹⁵ prefer to visualize ventricular fibrillation as a state in which the heart has been divided into a multiplicity of ectopic foci with numerous individually acting units.

Ionic Mechanisms and Fibrillation. The ionic basis for changes in the electrical properties of the heart during fibrillation is now available. In 1935 Kehring and Hooker¹⁶ observed a transient

rise of K in coronary sinus blood of fibrillating dog hearts. They concluded that readily diffusible K ions were associated with the phenomenon of ventricular fibrillation. More recently these findings have been confirmed and extended in our own laboratory working with isolated rabbit atria. In the initial study⁷ data were obtained which suggested that fibrillation only began when the rate of net loss of K and gain of Na exceeded a critical one. For K this rate was of the order of 2.0 to 2.5 mmoles per kgm wet tissue per minute. The rate for Na could not be established with accuracy but it appeared to be considerably higher.

Later isotope studies^{8,9} revealed that the net loss of K resulted from an increased efflux of K ; influx was slightly increased or unaffected. The net gain of Na that occurred resulted from a marked increase in the inward movement of Na even though outflow was also increased to some extent. Since K influx was not greatly changed during fibrillation it was reasoned that an increased membrane permeability to K was not the prime factor involved. It was postulated that a more likely cause was the marked increase in the inward movement of Na with K leaving the tissue in exchange.

Several other points of interest were also established. The changes in Na and K fluxes were only transient in nature with flux rates returning to normal even though fibrillation continued. The changes in Na transport persisted for longer periods of time than those for K . This indicated that the increased membrane permeability to Na was involved primarily with the initiation of fibrillation. However these studies did not rule out the possibility that an increased Na permeability was a factor of importance in the maintenance of the arrhythmia.

Calculations revealed that the positive charge entering the atria as Na exceeded that which was lost as K . This suggested that permeability to an anion, possibly Cl , was also increased. Studies¹⁰ using Cl^{36} showed that the net gain of positive charge (difference between Na uptake and K loss) was balanced by a net gain of Cl .

These findings led to the following rather crude picture of the nature of ionic events accompanying fibrillation. The onset of fibrillation probably results from a marked increase in the inward

movement of Na^+ ions. If the atrial cells are to respond repeatedly and at a fast rate there must be some device which rapidly inactivates the inward Na^+ movement. That is the process of Na^+ inactivation (see Chapter V) must be greatly accelerated if the arrhythmia is to continue. An increase in the outward movement of K^+ or inward movement of Cl^- or a combination of both would be possible mechanisms. The outward movement of K^+ may contribute initially to the inactivation process whereas the increased inward movement of Cl^- may make a predominant contribution to the process of inactivation once the arrhythmia has persisted for some time. Recently it has been shown that Ca^{++} ions determine the extent to which the inward movement of Cl^- and outflow of K^+ contribute to the inactivation process.³¹ At high Ca^{++} concentrations the inflow of Cl^- is the predominating factor.

According to this scheme the antiarrhythmic agents should act by depressing the inward movement of Na^+ . The available evidence³ to date would indicate that such is the case.

Metabolic Events and Fibrillation: There have been a limited number of studies on changes in oxygen consumption and substrate utilization during ventricular fibrillation.³²⁻³⁴ The evidence indicates that as long as the ventricles are supplied with blood by an extracorporeal pump oxygen consumption, extraction of essential substrates and the content of energy rich compounds are well within normal limits.

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Chapter V

PERSPECTIVES

At the current rate of progress there is little doubt that in the foreseeable future we may know the detailed chemistry of most biological processes including muscle contraction. When all the chemistry is known we will still be unable to understand how the processes are effected and how the high state of cellular organization is maintained without a much greater emphasis on physical mechanism. In the present monograph the authors have carried the reader from the macroscopic through the microscopic then to the molecular and submolecular level in an attempt to understand the processes associated with contraction of the normal and failing heart.

We have adhered to the classical view of Frank and Starling that all phenomena associated with congestive heart failure can be explained by the concept of excessive stretch of the myocardial fiber resulting from increased demands placed upon the heart. Our thesis is that heart and skeletal muscle contract to perform work by an interaction between sites on interdigitating myofilaments in the sarcomere. This interaction appears to be between ATPase sites on the thick myosin filament in the A band and ATP bound to actin in the thin I band filament. The chemical correlate of the Frank Starling principle was pictured as a diminishing interaction resulting from gradual withdrawal of the I band filament from the A band. The latter was due to excessive stretch of the myocardial fiber.

The role of Ca ions to enhance and maintain interaction was emphasized. A decrease of Ca ions as is known to occur in congestive failure should lead to less interaction and weaker contractions. We presented evidence that digitalis acting at the

membrane increased the available Ca in the environs of the contractile filaments thereby restoring the normal rate of interaction. It was pointed out that with excessive stretch interaction would disappear in some fibers. When a sufficient number of fibers were involved intractable heart failure should appear and the beneficial effects of digitalis should be lost.

We briefly reviewed some of the recent advances in our knowledge of fibrillation, a phenomenon intimately associated with heart failure. Apparently this arrhythmia results from the establishment and maintenance of an abnormal membrane permeability to Na ions. Here again Ca plays a very crucial role.

Many problems remain to be solved. The most important is the nature of the physicochemical mechanism involved in the development of hypertrophy. It is hoped that with the application of the powerful physical techniques available that we will soon know more of this intriguing phenomenon.

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